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An Analysis of Morphometric Characters of Greenland Halibut (*Reinhardtius hippoglossoides*) in the Northwest Atlantic Using a Multivariate Analysis of Covariance

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Bowering, W. R. 1988. An analysis of morphometric characters of Greenland halibut (*Reinhardtius hippoglossoides*) in the Northwest Atlantic using a multivariate analysis of covariance. Can. J. Fish. Aquat. Sci. 45: 580–585.

Eleven morphometric characters were measured on samples of Greenland halibut (*Reinhardtius hippoglossoides*) from eight different areas of high Greenland halibut abundance throughout the Northwest Atlantic from Davis Strait to the northern Grand Bank. These data were then analysed using a multivariate analysis of covariance technique with the aim of using the results to delineate Greenland halibut stocks throughout the study area. The results suggested that each area examined may very well support a separate stock. However, given the state of knowledge of this resource, this was not believed to be truly the case. Alternative explanations for the results are discussed.

L'auteur a mesuré onze caractères morphométriques d'échantillons de flétans du Groenland (*Reinhardtius hippoglossoides*) provenant de huit zones différentes de forte abondance réparties dans tout le nord-ouest de l'Atlantique, du détroit de Davis à la partie nord du Grand Banc. Les données ont été analysées par techniques de covariance à variables multiples dans le but d'en utiliser les résultats pour déterminer les limites des stocks de flétan du Groenland dans la zone étudiée. Les résultats obtenus portent à croire que chacune des zones examinées pourrait abriter un stock distinct, mais étant donné les connaissances que nous avons actuellement de cette ressource, il ne semble pas que cela soit vraiment le cas. L'auteur présente d'autres possibilités pour expliquer les résultats obtenus.

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Greenland halibut (*Reinhardtius hippoglossoides*) in the Northwest Atlantic are widely distributed along the west coast of Greenland, into Davis Strait, and down the eastern Canadian coast from Baffin Island to the Grand Banks of Newfoundland (Bowering 1983) (Fig. 1). They are also found in commercial abundance in the Gulf of St. Lawrence and Fortune Bay (Bowering 1983). Greenland halibut is one of the major groundfish resources in the Northwest Atlantic where the total allowable annual catch is now at a level of 135 000 metric tons.

Effective management of such an important resource requires extensive knowledge of the biological characteristics of the species and, in particular, the separation of the species into discrete spawning units or stocks. Morphometric measurements are one of the more common methods used for identifying fish stocks and have been widely used for differentiating stocks of many species (e.g. see Sharp et al. 1978; Casselman et al. 1981; Ihssen et al. 1981; Almeida 1982; Davidson et al. 1982).

In this presentation, morphometric measurements of Greenland halibut of the Canadian Northwest Atlantic were analysed for differences among eight areas. The objective of the study was to delineate stocks from comparisons of samples from these areas.

Materials and Methods

All materials used in this study were collected by Canadian Newfoundland-based research vessels chartered for research purposes, generally those involved in stratified-random or fixed station biomass surveys for groundfish in all areas of the Northwest Atlantic. These cruises took place at much the same time of the year for each individual area; consequently, it was not possible to collect samples for the same season and year for the whole study area. All vessels were otter trawlers using various types of bottom otter trawls or, in some cases, shrimp trawls with similar small-mesh liners in the codend to avoid the escapement of small fish.

All specimens were collected during 1978–80. Eight different test areas (Fig. 1) were selected where a fishery for Greenland halibut has occurred historically. The eight test areas and sample sizes for these areas are presented in Table 1. Fish were frozen in plastic bags at sea and returned to the laboratory for detailed analysis. Upon thawing, all morphometric measurements were made with vernier calipers except for total, standard, and fork lengths which were measured on a standard 1-cm-interval measuring board.

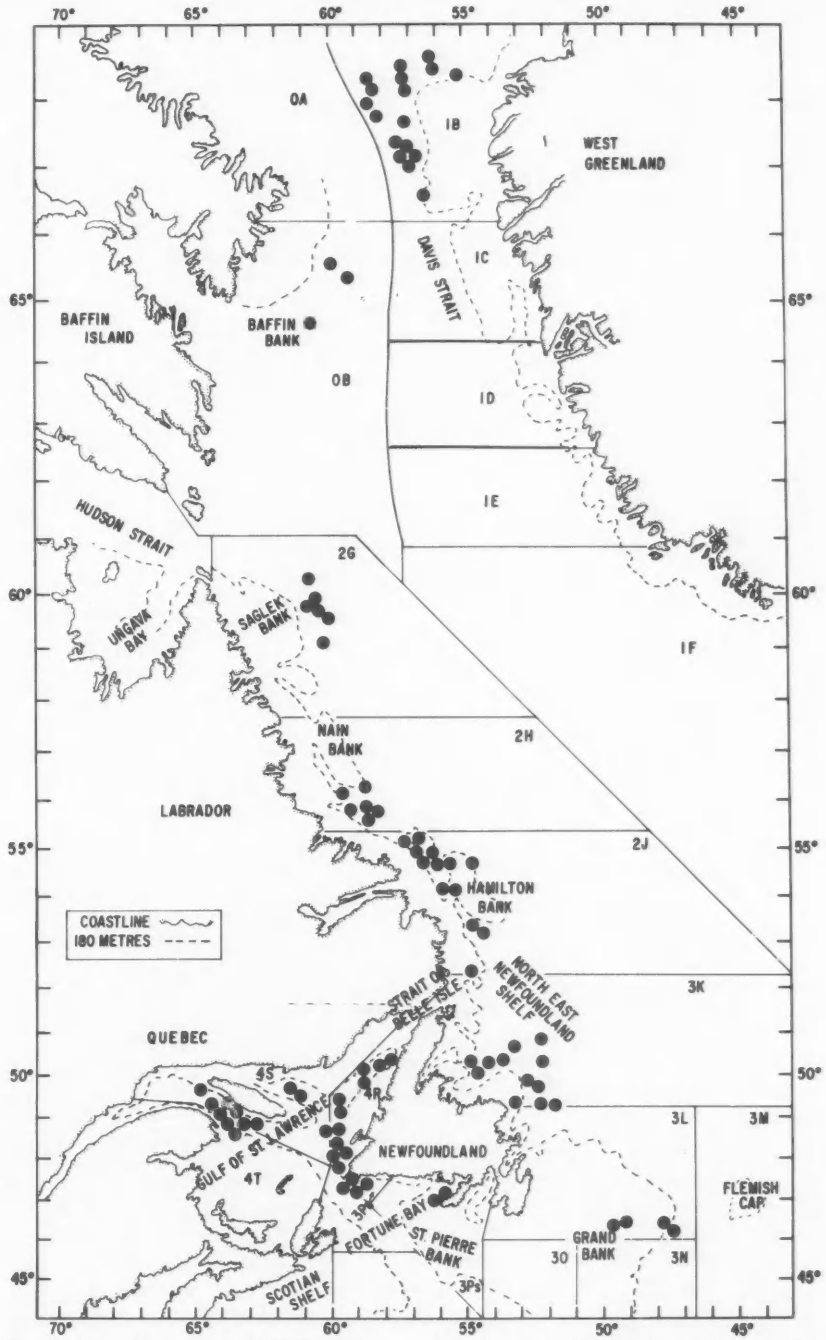

FIG. 1. Major place names and NAFO divisions mentioned in the text. Solid circles represent sampling locations for morphometric data.

The following 11 morphometric characters, designated y_i where $i = 1, 2, \ldots, 11$, were measured on each individual: fork length, total length, head length (y_3), snout length (y_4), upper jaw length (y_5), interorbital width (y_6), predorsal length (y_7), prepectoral length (y_8), preventral length (y_9), preanal length (y_{10}), and standard length (y_{11}). To avoid undue inflation of correlation between characters, measurements fork length y_1 and total length y_2 were replaced in the analysis by the differences y_1 = (fork length − standard length) and y_2 = (total length − standard length).

TABLE 1. Numbers of specimens collected from eight locations with identification number used in statistical analysis.

Location	Sample No. used in statistical analyses	Number sampled
West Greenland	1	134
Baffin Bank	2	159
Saglek Bank	3	165
Nain Bank	4	280
Hamilton Bank and Northeast Newfoundland Shelf	5	175
Northern Grand Bank	6	154
Fortune Bay	7	150
Gulf of St. Lawrence	8	166

Statistical Analyses and Results

All statistical tests were done at the 5% level of significance unless otherwise stated. Population means were not compared by analysing characters individually, i.e. by a univariate analysis of variance (ANOVA), as "a series of univariate statistical analyses carried out separately for each of the variables is, in general, not adequate as it ignores the correlations among variables" (Kshirsagar 1972). In comparing population means, consideration was also given to the fact that individuals vary in size within populations, so some correction for length was warranted (Bliss 1970; Misra and Ni 1983). Equality of mean vectors of populations was therefore tested by the multivariate analysis of covariance (MANCOVA) using the program of Misra and Ni (1983). By employing standard length as the covariate, MANCOVA compares population mean vectors which are adjusted for different values of the standard length (Morrison 1976; Misra and Ni 1983). All measurements were transformed to common logarithms for these analyses, for the following reasons: (1) in the MANCOVA it is assumed that the responses are multinormal (Morrison 1976); (2) it is assumed that the variance–covariance matrices are homogeneous; (3) multivariate normality is more closely approximated by logarithms than the original variables (Bliss 1967; Pimentel 1979); (4) in the MANCOVA, sample mean vectors are adjusted on the assumption of linear relationships with the covariate; logarithmic transformations should satisfy a test of linearity (Pimentel 1979), as indeed was our finding (reported below); (5) the convention is to use common logarithm (Pimentel 1979). Only complete specimens, i.e. specimens for which all eleven measurements were available, were employed in the statistical analysis, as "missing observations virtually destroy morphometrics" (Pimentel 1979). Samples from the eight test areas were designated 1 through 8 for statistical analysis, as shown in Table 1. Table 2 shows means and ranges of each of the 10 characters for each sample. Large overlaps of ranges of all variates and the covariate between samples occurred (Table 2). These suggested the following: (1) a measurement with large overlap is unlikely to aid in separating samples (see Bliss 1970); this reinforced the use of the MANCOVA in preference to analyses at the univariate levels; (2) large overlaps of standard length between samples provided additional support for a reasonable covariate (see Snedecor and Cochran 1967).

The MANCOVA model assumes that the within-sample vector of coefficients of regression of the variates on the covariate are similar for all areas. The null hypothesis of no differences of these regression coefficients (weighted by the SS of the covariate) among areas was tested and accepted ($P > 0.9$). MANCOVA compares vectors of means of variates, adjusted for variations in the values of the covariate by employing the linear relationships of the variates with the covariate. It is obvious

TABLE 2. Test areas and transformed means and ranges of 10 characters. All measurements were transformed to common logarithms.

	Location							
Character No.	West Greenland	Baffin Island	Saglek Bank	Nain Bank	Hawke Channel Funk Island Deep	Northern Grand Bank	Fortune Bay	Gulf of St. Lawrence
1	1.663 1.519–1.826	1.643 1.505–1.778	1.700 1.580–1.806	1.676 0.699–1.792	1.633 1.477–1.763	1.604 1.462–1.716	1.652 1.477–1.771	1.696 1.519–1.785
2	1.729 1.556–1.914	1.705 1.556–1.857	1.775 1.633–1.892	1.749 1.079–1.898	1.707 1.544–1.833	1.660 1.544–1.778	1.718 1.580–1.839	1.763 1.580–1.851
3	1.984 1.851–2.121	1.966 1.839–2.107	2.035 1.863–2.134	2.014 1.839–2.137	1.978 1.845–2.117	1.927 1.839–2.000	1.964 1.826–2.090	2.005 1.858–2.100
4	1.519 1.350–1.769	1.500 1.367–1.709	1.561 1.375–1.668	1.542 1.354–1.684	1.527 1.391–1.692	1.468 1.352–1.563	1.517 1.384–1.652	1.526 1.378–1.639
5	1.645 1.491–1.800	1.618 1.494–1.769	1.689 1.516–1.805	1.671 1.501–1.801	1.632 1.476–1.790	1.576 1.483–1.649	1.623 1.477–1.761	1.647 1.502–1.750
6	1.108 0.964–1.303	1.107 0.934–1.270	1.153 0.959–1.320	1.135 0.908–1.310	1.150 0.954–1.327	1.036 0.845–1.179	1.114 0.949–1.301	1.117 0.886–1.260
7	1.662 1.519–1.833	1.634 1.415–1.770	1.701 1.531–1.813	1.681 1.491–1.806	1.663 1.519–1.785	1.614 1.519–1.690	1.643 1.519–1.756	1.664 1.519–1.763
8	1.963 1.839–2.107	1.946 1.813–2.090	2.012 1.839–2.117	1.992 1.813–2.117	1.964 1.826–2.104	1.911 1.826–1.996	1.947 1.792–2.076	1.983 1.839–2.079
9	1.944 1.813–2.100	1.930 1.785–2.086	1.988 1.807–2.104	1.972 1.785–2.121	1.949 1.813–2.104	1.898 1.792–1.978	1.922 1.756–2.053	1.962 1.813–2.069
10	2.092 1.959–2.255	2.074 1.908–2.190	2.143 1.982–2.246	2.123 1.908–2.253	2.094 1.944–2.262	2.061 1.914–2.143	2.097 1.935–2.220	2.112 1.940–2.225

TABLE 3. Individual characters contributing significantly (marked "×") to paired comparisons of samples.

Sample pair	Character No. 1	2	3	4	5	6	7	8	9	10
(1,2)										
(1,3)										
(1,4)										
(1,5)						×				
(1,6)			×		×					
(1,7)			×		×			×	×	
(1,8)					×		×			
(2,3)						×				
(2,4)										
(2,5)							×			
(2,6)			×		×	×				
(2,7)			×					×	×	
(2,8)						×				
(3,4)										
(3,5)						×			×	
(3,6)			×		×					
(3,7)			×					×		
(3,8)					×					
(4,5)						×				
(4,6)			×		×					
(4,7)			×		×			×	×	
(4,8)					×		×			
(5,6)				×	×	×				
(5,7)			×			×	×	×	×	
(5,8)	×	×		×	×	×	×	×	×	
(6,7)									×	
(6,8)		×								×
(7,8)			×			×				

TABLE 4. Results of canonical discriminant function analysis for morphometric data on Greenland halibut.

Canonical discriminant function	Canonical correlation	Eigenvalue	Proportion	PR > F
1	0.6422	0.7022	0.4139	<0.0001
2	0.5052	0.3428	0.2021	<0.0001
3	0.4897	0.3155	0.1860	<0.0001
4	0.4253	0.2209	0.1302	<0.0001
5	0.2817	0.0862	0.0508	0.0001
6	0.1574	0.0254	0.0150	0.0001
7	0.0589	0.0035	0.0021	0.4429

therefore that a comparison of means by the MANCOVA would be more effective than by MANOVA (which ignores the covariate) only when the linear relationships of the variates with the covariate exist. The hypothesis of independence of the variates on the covariate was tested by the union intersection procedure (Morrison 1976) and was rejected ($P < 0.0001$). This indicated a strong linear relationship of the dependent variates with the covariate implying that the residual variation of $y_i (i = 1,2,\ldots,10)$ about the sample regression would be considerably smaller than the variation around the mean of a sample when the covariate is ignored. The null hypothesis of equality of the

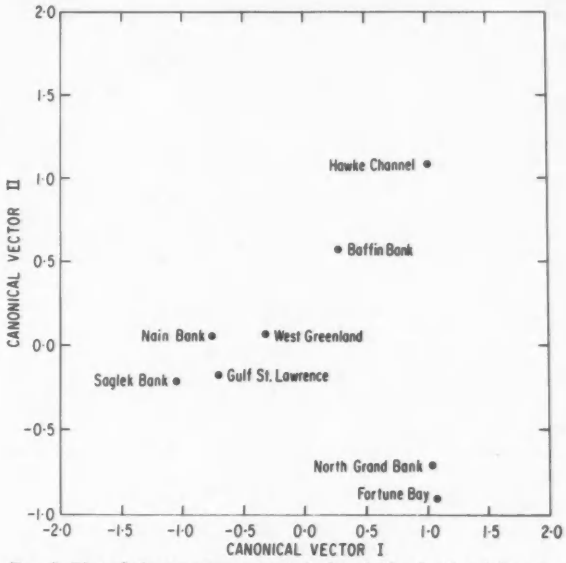

FIG. 2. Plot of class means on canonical variables for the eight areas examined for CVI against CVII.

adjusted mean vectors of populations was then tested by the MANCOVA (Morrison 1976). This hypothesis was rejected ($P < 0.0001$), indicating thereby that the vectors of adjusted area means were not all equal. Note that (1) rejection of the null hypothesis does not indicate which areas or characters or character–area combinations may have led to the rejection and (2) a difference in mean vectors of areas may not be attributable to an individual variate but to a linear compound of variates (Bliss 1970; Morrison 1976). These points imply that a contrast among area means can be significant and yet the difference may not be shown by individual characters. However, a search for all significant response–sample combinations was not carried out. Instead, comparisons were restricted to pairs of areas and to individual characters. Following the union intersection procedure (Morrison 1976), 95% simultaneous confidence intervals were constructed to test the significance of the contributions of individual characters to the difference between adjusted mean vectors of pairwise areas. The null hypothesis of no significant contribution of a character was accepted if the simultaneous confidence interval included a zero value. MANCOVA showed that all paired comparisons of the eight areas were significant (all $P < 0.001$). In Table 3, individual characters which contributed to the difference between area of each pair are marked "×." All characters contributed to at least one between-area difference, but characters 1, 2, 4, and 10 contributed less than the others. The results of a canonical discriminant function analysis (Table 4) indicated that most of the variance in the data was accounted for by the first four canonical discriminant functions with about 62% accounted for in CVI and CVII. A plot of CVI class means aginst CVII class means (Fig. 2) suggests some geographical gradient in that the more southerly areas (i.e. North Grand Bank and Fortune Bay) were more closely related than to the more northerly areas and the Gulf of St. Lawrence. However, no real geographic trend could be established.

Discussion

Results of this analysis of morphometric characters imply that each of the test areas examined is occupied by a biologically discrete population of Greenland halibut. Other studies of stock discrimination have suggested that the Gulf of St. Lawrence and Fortune Bay support different spawning stocks but generally consider the Greenland halibut population from Davis Strait to the northern Grand Bank to be a single spawning stock. The results of such studies can be found in Misra and Bowering (1984) using meristics, Fairbairn (1981) using biochemical genetics, Khan et al. (1982) using blood protozoa as biological tags, and Bowering (1984a) using external tagging experiments. Furthermore, biological studies on distribution patterns (Templeman 1973; Bowering 1984b), age and growth and sexual maturity (Bowering 1983), and spawning and nursery areas (Smidt 1969) all concluded that the one stock existed from Davis Strait to the northern Grand Bank. A review of all these studies can be found in Misra and Bowering (1984); therefore, it will not be repeated here. Given that all these published results reached the same conclusions, it is puzzling that the morphometric analysis presented here implies that there are at least six highly discrete spawning stocks from Davis Strait to the northern Grand Bank. This is particularly perplexing because these are exactly the same fish used in this study as were used in the meristic study by Bowering and Misra (1984) and the blood protozoa study by Khan et al. (1982).

In their study on morphological differentiation in ciscoes (Coregoninae), Todd et al. (1981) demonstrated that not only genetic but also environmental factors influence morphological variation strongly. If morphological differences between populations are primarily the result of environmental effects during development, then the populations should be considered as one stock. A theoretical view of the role of natural selection in explaining genetic properties of metric characters was given by Falconer (1981) and an investigation of the question of what constitutes a unit stock made by Gulland (1983). Morphometric characters of many organisms probably have intermediate optima, with large additive as well as nonadditive components of genotypic variance. A difference may therefore not be clear evidence of genetic separations of populations. A further explanation is that there may have been clinal variation which of course does not require the isolation of breeding stocks, but merely something less than complete mixing. For example, fish from the northern Grand Bank may be more likely to breed with other fish from the northern Grand Bank rather than those from West Greenland. To take discontinuous samples from such a cline, if it exists, would likely show all areas as being different. This is particularly true with an analysis technique as sensitive as that used here and with large sample sizes. This is rather evident considering the relatively low eigenvalues, and yet, every area was highly significantly different from every other area. To test for this, however, would require intensive resampling of the whole area studied with care not to allow for discontinuity in sampling. This is not considered practical here.

Conclusions

Given that the same fish were used for several other studies which concluded that there was a single population from Davis Strait to the Grand Bank, there would appear to be factors that cause morphometric differences other than the existence of discrete spawning populations. Except for the existing overwhelming evidence for a single stock from Davis Strait to the Grand Bank, strong (yet erroneous) conclusions may well have been drawn regarding the stock composition of this resource. It is important to realize, therefore, that using a single traditional method for stock identification work such as morphometric analysis is probably not enough on its own to reach definite conclusions regarding stock delineation.

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Estimating Stock Composition of Migrating Juvenile Fraser River (British Columbia) Sockeye Salmon, *Oncorhynchus nerka*, Using Parasites as Natural Tags

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Bailey, R. E., L. Margolis, and C. Groot. 1988. Estimating stock composition of migrating juvenile Fraser River (British Columbia) sockeye salmon, *Oncorhynchus nerka*, using parasites as natural tags. Can. J. Fish. Aquat. Sci. 45: 586–591.

Simulated mixtures of juvenile sockeye salmon (*Oncorhynchus nerka*) were constructed using parasite data to represent proportionally the major component stocks of Fraser River and Lake Washington sockeye migrating within the Strait of Georgia, British Columbia, in 1982–84. Samples of migrating juveniles were also collected from Bedwell Harbour, South Pender Island, British Columbia, each year and analyzed for parasites and stock composition. The compositions of simulated and sample mixtures were estimated using a maximum likelihood stock composition model. Simulated mixture compositions were accurately estimated for most stocks for all year-classes. When significant misassignment occurred between stocks, the stocks were analyzed as a complex using the allocate-sum procedure. Sample mixture estimates correctly identified the dominant stock for each year-class, although for 1984 the dominant group was determined as a complex of three stocks because the individual stocks were not distinguishable. The results indicate that it is feasible to use parasites as natural tags to estimate stock compositions of migrating juvenile sockeye salmon in the Strait of Georgia.

Les auteurs ont simulé des groupes de saumons nerkas juvéniles (*Oncorhynchus nerka*) à partir de données sur les parasites afin de représenter de façon proportionnelle les principales composantes de stock des saumons nerkas du fleuve Fraser et du lac Washington qui étaient en migration dans le détroit de Géorgie (Colombie-Britannique) de 1982 à 1984. Des juvéniles en migration ont aussi été prélevés à chaque année à Bedwell Harbour, sur l'île South Pender (Colombie-Britannique) et fait l'objet d'une analyse portant sur la présence de parasites et la composition des stocks. Les compositions des groupes simulés et des échantillons ont été estimées à l'aide d'un modèle de la composition des stocks par maximum de vraisemblance. Les compositions des groupes simulés ont été estimées avec précision, ceci pour la majorité des stocks de toutes les classes annuelles. Dans les cas d'écarts significatifs entre les stocks, ceux-ci ont été analysés sous la forme d'un complexe en faisant appel à la procédure de répartition–somme. Les estimations des groupes échantillons ont permis d'identifier avec exactitude le stock dominant de chacune des classes annuelles, même si le groupe dominant de 1984 a été jugé être un complexe de trois stocks, les stocks individuels ne pouvant être distingués. Les résultats obtenus montrent qu'il est possible d'utiliser les parasites comme marqueurs naturels permettant d'estimer la composition des stocks des juvéniles de saumons nerkas en migration dans le détroit de Géorgie.

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The Fraser River in British Columbia, Canada, is among the most important producers of sockeye salmon (*Oncorhynchus nerka*) along the west coast of North America and contains approximately 50–60 stocks that spawn in tributaries of about 22 lakes, although approximately 90% or more of the annual smolt output of the Fraser River system originates from seven or eight lakes (see Table 2). Sockeye from this river system typically spend 1 yr in nursery lakes after emergence before migrating to sea as smolts. Upon reaching the estuary the smolts migrate northwestward through the Strait of Georgia to reach the Pacific Ocean where they spend 1, 2, or 3 yr before returning to their home rivers to spawn.

Adults of the major populations or stocks of Fraser River sockeye have been distinguished by scale characteristics (Henry 1961), althought this technique has not been applied to determining stock origins of juvenile stages of these fish. The pioneering studies of Margolis (1956, 1957, 1963) and Kabata (1959, 1963) showed that parasites can be used as natural tags to differentiate fish populations. In their approach, two or three fish stocks or stock groups are separated on the basis of one or two unique parasite species present in one stock or stock group and absent in another.

Groot et al. (1984) reported preliminary results indicating that Fraser River juvenile sockeye stocks have different parasite assemblages, and Bailey and Margolis (1987) presented comprehensive parasitological data for the stocks from the Fraser River system. Only one parasite, *Ergasilus nerkae*, was unique to one of the stocks (Seton Lake) from this system, precluding the use of the Margolis and Kabata approach for identification of individual Fraser River stocks. Konovalov (1971) proposed a multivariate stock classification key for sockeye based on scale and parasite data. Fournier et al. (1984) developed a more ver-

satile multivariate maximum likelihood model that can employ several biological markers to separate stocks. This model was successfully employed by Wood et al. (1987b) to differentiate various stocks of maturing sockeye captured during their homing migration off southeastern Alaska, using scale, parasite, meristic, and electrophoretic data.

In this paper we examine if differences in parasite assemblages of Fraser River sockeye juveniles can be used to resolve the stock composition of sockeye smolts captured during seaward migration in the Strait of Georgia. The analysis employs parasite data of Bailey and Margolis (1987) for Fraser River juvenile sockeye salmon and the mixed fishery stock composition estimation procedures described by Fournier et al. (1984) and Wood et al. (1987a).

Materials and Methods

Choice of Stocks

As previously noted, about 90% of the sockeye smolt output of the Fraser River in any given year originate from seven or eight lakes (Fig. 1). As these smolts emigrate through the Strait of Georgia, they may mix with smolts from other sockeye stocks, e.g. Lake Washington, Washington State. Escapement data for the parental brood years were used to determine the six (1983, 1984) or seven (1982) stocks that were likely to contribute greater than 95% of the total smolt population within the Strait of Georgia in a particular year. We decided to treat Harrison Lake and Lillooet Lake stocks as one group because comparison of parasite data for presmolt juvenile and adult escapement samples from both systems indicated that more than 70% of Birkenhead River sockeye fry migrate through Lillooet Lake to Harrison Lake, where they rear. J. Cave (Pacific Salmon Commission, 600-1155 Robson St., Vancouver, B.C. V6E 1B9, pers. comm.) estimated that 70–95% of Birkenhead fry rear at least partially in Harrison Lake.

Choice of Parasites

For a parasite to be suitable as a biological tag for juvenile sockeye during their early marine life, it must infect the young salmon in the home or rearing system, remain on or within the host for at least 2–4 wk after entering seawater, and show differences in abundance among stocks. Thirteen freshwater parasite species reported by Bailey and Margolis (1987) fulfilled these criteria (Table 1). Of these, *Myxidium salvelini, Phyllodistomum umblae, Neoechinorhynchus* spp., and *Salmincola californiensis* persist for a minimum of 2–4 wk after entry to seawater (R. E. Bailey and L. Margolis, unpubl. data) and therefore are potentially useful as natural tags for juvenile sockeye salmon. *Philonema agubernaculum* persists for at least 1 yr after smolting of sockeye (R. E. Bailey and L. Margolis, unpubl. data). *Diplostomum* sp. (body cavity), dilepidid cysticercoids, and *Ergasilus nerkae* were observed parasitizing juvenile salmon in the Strait of Georgia, indicating that these parasites can survive for at least 1 mo after sea entry of the smolts. The remaining parasites listed in Table 1 have been observed in returning adult sockeye and seem to persist for the duration of the salmon's ocean life.

Myxidium salvelini was not employed as a tag because its prevalence in Harrison/Lillooet Lake outmigrants was not known. It was observed in 55% of presmolts from Lillooet Lake but was not observed in a sample of presmolts from Harrison Lake.

Stock Composition Analysis

Monte-Carlo simulations

To test the potential reliability of stock composition estimates using parasite data, stock composition was estimated initially for simulated mixtures consisting of known proportions of sockeye from six or seven "stocks" or stock groups. These proportions were selected to reflect estimated contributions by the stocks to the Strait of Georgia juvenile sockeye population in the years 1982–84. The relative contribution of each stock or stock group to the overall mixture of smolts in the Strait of Georgia was estimated from the relative escapements of the parents, assuming that the production of smolts per spawner for each lake was equal. The six (1983, 1984) or seven (1982) stocks that accounted for more than 95% of the predicted sockeye progeny in the noted years were selected. The mixture of smolts within the Strait of Georgia was then assumed to consist of only those stocks (Table 2).

For each year-class, a hypothetical mixture of 500 fish was generated using the stock composition simulation model described by Fournier et al. (1984) and parasite prevalence data from Bailey and Margolis (1987). The proportions of each stock in the hypothetical mixtures for each year-class are given in Table 2. All parasites were coded as present or absent except for *Diphyllobothrium* spp. plerocercoids for which three categories were employed: absent, one to three, or four or more worms present. A new simulated learning sample of 200 fish per stock was generated simultaneously with the hypothetical mixture. These learning samples were randomly based on the observed prevalences of infection by parasites in the original learning samples (n = 50–244). This procedure ensured that the effect of random sampling error was simulated in generating both the mixture and learning samples. Stock composition of the mixture was then estimated using the model described by Fournier et al. (1984), and the entire process was repeated a total of 100 times per year-class. When considerable missassignment occurred between stocks, their relative contributions were summed and analyzed collectively, following the allocate-sum procedure of Wood et al. (1987a). Hypothetical mixtures and learning samples employed in the simulations were larger than the samples available for test fishery analyses to demonstrate the capability of the model under optimal conditions.

Test fishery samples

Three samples of juvenile sockeye salmon (ranging in numbers from 108 to 199), one sample for each of the years 1982–84, were collected from Bedwell Harbour, South Pender Island, British Columbia, during the annual smolt emigrations (Groot et al. 1985). These fish were subjected to complete necropsies for protozoan and metazoan parasites as described by Bailey and Margolis (1987). To estimate the variance of stock composition estimates for a test fishery sample, a subsample of equivalent size to the test fishery sample was generated (with replacement) from the original sample (bootstrapping) (Efron 1982). Simulated learning samples of sizes equal to the original learning samples were also generated, and the stock composition of the bootstrapped mixture was determined as already described. Fish originating from stocks not represented in the learning samples and that may have been present in test fishery samples would have been allocated to the most parasitologically similar stock included in the learning samples. Therefore, the estimates for some stocks may have been biased by the misallocation of those fish. Such fish were assumed to contribute less than 5% of any mixture.

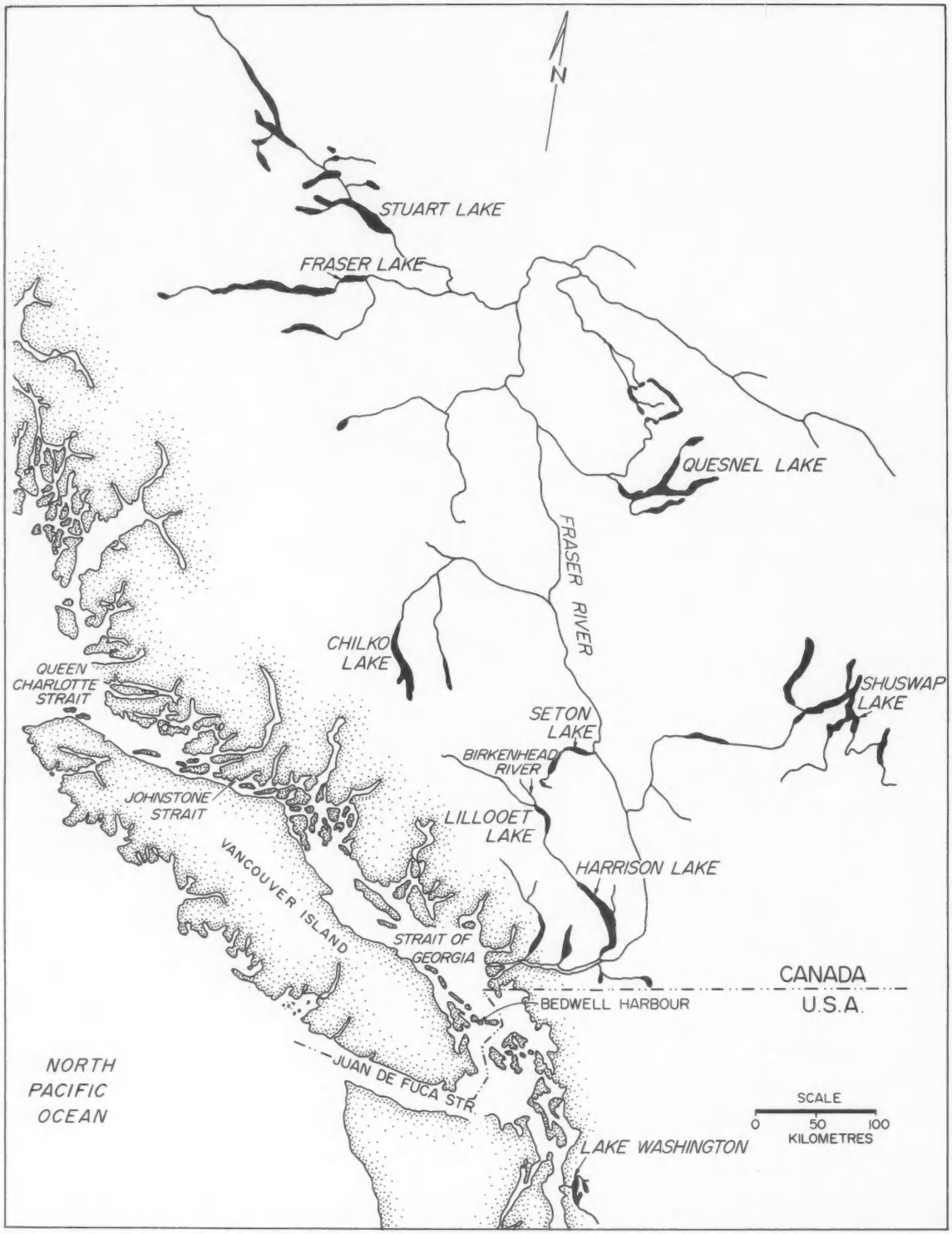

FIG. 1. Sockeye salmon nursery lakes sampled in this study.

TABLE 1. Parasites potentially suitable as biological tags for migrating juvenile sockeye salmon.

Parasite	Site of infection
Myxidium salvelini	Urinary bladder, kidney
Myxobolus neurobius	Brain, spinal column
Diplostomum sp. metacercaria	Eyes
Diplostomum sp. metacercaria	Body cavity
Tetracotyle sp. metacercaria	Viscera, pericardium, kidney
Phyllodistomum umblae	Ureters of kidney, urinary bladder
Diphyllobothrium plerocercoids	Viscera, mesenteries
Dilepidid cysticercoid	Liver
Philonema agubernaculum	Swimbladder, body cavity
Philonema oncorhynchi	Swimbladder
Neoechinorhynchus spp.	Caeca, small intestine
Ergasilus nerkae	Gills
Salmincola californiensis	Gills, external surfaces

Results

Monte Carlo Simulations

The true proportions of all populations fell well within the 95% confidence limits (2 SD) of their estimated contributions (Table 2, column 2). The shapes of distributions of estimates were approximately normal when mean estimates were greater than 0.1 and were highly skewed to the left when mean estimates were less than 0.05. When significant misassignment occurred, distributions tended to be somewhat wider, although remaining approximately normal if the mean estimate exceeded 0.1. Where the allocate-sum procedure was employed, estimates tended to be normally distributed in a fairly narrow range, i.e. in 1983 the range of estimates for Harrison/Lillooet/Shuswap was within 0.06–0.22, although 90% of the estimates were between 0.08 and 0.19. When population estimates are normally distributed, ± 2 SD represent a 95% confidence interval.

Proportionally higher standard deviations were observed for Harrison/Lillooet, Seton, and Shuswap Lake stocks for 1982 and Harrison/Lillooet and Shuswap Lake stocks for 1983 and 1984, indicating some degree of misassignment among these stocks. When Harrison/Lillooet and Shuswap Lake stocks were treated as a complex and analyzed together following the allocate-sum procedure (Wood et al. 1987a), their new standard deviation was reduced from 0.042 and 0.032 to 0.030 for 1983 and from 0.040 and 0.040 to 0.016 for 1984. However, no reduction was noted for 1982. When Seton Lake was also included for 1982, the combined standard deviation decreased slightly from 0.039, 0.026, and 0.023 to 0.023. The reduced standard deviations noted for the combined estimates clearly illustrate the misassignment between the above stocks; therefore, these stocks should be analyzed collectively because

TABLE 2. Estimated compositions (± 1 SD) of simulated and sample mixtures for 1982, 1983, and 1984 year-classes of juvenile sockeye in the Strait of Georgia, British Columbia.

Stocks	Actual proportion in simulated mixture[a]	Estimated proportion in simulated mixture	Estimated proportion in test fishery sample
1982			
Chilko	0.428	0.428 ± 0.026	0.799 ± 0.055
Fraser	0.064	0.055 ± 0.013	0.113 ± 0.041
Harrison/Lillooet	0.147	0.145 ± 0.039	0.011 ± 0.025
Seton	0.024	0.022 ± 0.026	0.003 ± 0.010
Shuswap	0.013	0.022 ± 0.023	0.054 ± 0.034
Stuart	0.015	0.017 ± 0.011	0.001 ± 0.004
Lake Washington	0.309	0.311 ± 0.028	0.019 ± 0.028
Harrison/Lillooet, Seton, Shuswap comb.	0.184	0.189 ± 0.023	0.068 ± 0.036
1983			
Fraser	0.029	0.032 ± 0.011	0.005 ± 0.010
Harrison/Lillooet	0.077	0.084 ± 0.042	0.093 ± 0.075
Quesnel	0.509	0.502 ± 0.027	0.630 ± 0.086
Shuswap	0.053	0.049 ± 0.032	0.120 ± 0.082
Stuart	0.259	0.259 ± 0.024	0.139 ± 0.047
Lake Washington	0.073	0.074 ± 0.017	0.013 ± 0.036
Harrison/Lillooet, Shuswap comb.	0.130	0.133 ± 0.030	0.213 ± 0.078
1984			
Chilko	0.060	0.059 ± 0.014	0.114 ± 0.035
Fraser	0.017	0.018 ± 0.007	0.039 ± 0.027
Harrison/Lillooet	0.104	0.108 ± 0.040	0.059 ± 0.056
Quesnel	0.007	0.004 ± 0.002	0.026 ± 0.017
Shuswap	0.743	0.739 ± 0.040	0.745 ± 0.055
Lake Washington	0.069	0.072 ± 0.016	0.017 ± 0.025
Harrison/Lillooet, Shuswap comb.	0.847	0.847 ± 0.016	0.804 ± 0.037

[a]Actual proportions were selected as best estimates of expected proportions in the test fishery sample based on parental escapement data.

estimates of their individual contributions may be inaccurate due to misassignment.

Test Fishery Samples

The results of analyzing the bootstrapped mixtures for 1982, 1983, and 1984 are presented in Table 2, column 3. For each year-class, the predicted dominant stocks were identified as such by the model, and for 1982 and 1983, these stocks were estimated to be present in significantly greater numbers than predicted (Table 2, column 3).

Estimates for the Harrison/Lillooet and Shuswap Lake populations were combined again for 1983 and 1984 and for Harrison/Lillooet, Seton, and Shuswap lakes for 1982. The standard deviation of the summed estimates was smaller than those for the Harrison/Lillooet and Shuswap Lake stocks alone for 1984, smaller than that of the Shuswap Lake stock alone for 1983, but larger than any of the three contributing stocks alone for 1982 (Table 2, column 3). The shapes of the distributions of estimates were similar to those encountered in the analyses of simulation results, approximately normal when mean estimates were greater than 0.10 and skewed toward 0 when mean estimates were less than 0.05. Ranges of estimates tended to be larger in test fishery examples.

Discussion and Concluding Remarks

Simulations

The reliability of stock composition estimates for the simulated mixtures of juvenile sockeye salmon for 1982, 1983, and 1984 year-classes in the Strait of Georgia indicates that it is feasible to use parasites as natural tags to estimate the individual contributions of stocks to complex mixtures, provided sufficient markers are available. Previous studies on the use of parasites as biological tags relied on separating mixtures of two or three stocks or groups of stocks on the basis of one or two unique parasite species (e.g. Margolis 1956, 1957, 1963; Kabata 1959, 1963) or have used a single species of parasite to supplement a number of genetic and scale traits to increase separation (e.g. Wood et al. 1987b). The present study is the first in which stock compositions are estimated by employing the multivariate maximum likelihood mixture model of Fournier et al. (1984), using parasites alone as the natural tags.

The model was able to resolve the simulated mixtures with a high degree of accuracy. For all stocks, the true proportion fell within 2 SD of their mean estimated values. Fournier et al. (1984) and Wood et al. (1987a) both noted that the mixture model tended to overassign to stocks present at low proportions and underassign to stocks present at higher proportions. No significant under- or overassignment was observed in the present tests, which may be due to the large number of traits used to separate the stocks.

There was some difficulty in assigning fish to Harrison/Lillooet, Seton, and Shuswap Lake stocks in 1982 and to Harrison/Lillooet and Shuswap Lake stocks in 1983 and 1984. When these stocks were considered as a single group, i.e. using the allocate-sum procedure of Wood et al. (1987a), the standard deviation of the contribution of the combined stocks to the simulated mixture was reduced considerably. Wood et al. (1987a) noted that in such cases, estimates of the contributions of the individual stocks are not reliable, and the summed estimate should be used. The Harrison/Lillooet, Seton, and Shuswap Lake stocks are parasitologically very similar, although fish from Harrison/Lillooet and Shuswap lakes and from Harrison/Lillooet and Seton lakes may be easily distinguished electrophoretically at the phosphoglucomutase-1 (PGM-1) locus (C. Wood, Pacific Biological Station, Nanaimo, B.C. V9R 5K6, pers. comm.). Fish from Seton and Shuswap lakes, however, do not differ at this locus (C. Wood, pers. comm.).

Test Fishery Samples

Analyses of the bootstrapped mixtures from Bedwell Harbour clearly indicated that a large proportion of the fish present was from the predicted dominant stocks. For 1982 and 1983, the dominant stocks were present at a greater proportion than predicted. For 1984, the contribution of the Shuswap and Harrison/Lillooet lakes group was slightly below the predicted value, although still within 2 SD. Although these results appear to be reasonable, there are several potential difficulties that must be addressed prior to extensive use of the technique. The principal problem was a lack of sufficient learning samples for some stocks. The learning samples for Harrison/Lillooet lakes were augmented with parasite data from adult escapements because of a lack of adequate samples of juvenile fish. A potential lack of stability of the prevalence of some markers, both seasonally and annually (Bailey and Margolis 1987), or mixing adult and smolt learning samples, may bias estimates. Wood et al. (1987a) noted that stability of markers is a prerequisite for use of the technique when learning samples from corresponding years are unavailable. Many of the markers used in this study were stable in at least some of the stocks tested (Bailey and Margolis 1987). In cases where certain markers are not seasonally or annually stable, three samples of at least 50 fish each, taken at intervals during the smolt migrations, should have been examined for all stocks for every year-class to produce more accurate learning samples. This was not feasible in this study, so the analyses were undertaken with the available data.

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Predation Risk and Foraging in Juvenile Pink (*Oncorhynchus gorbuscha*) and Chum Salmon (*O. keta*)

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Magnhagen, C. 1988. Predation risk and foraging in juvenile pink (*Oncorhynchus gorbuscha*) and chum salmon (*O. keta*). Can. J. Fish. Aquat. Sci. 45: 592–596.

Habitat choice and foraging activities of juvenile pink (*Oncorhynchus gorbuscha*) and chum salmon (*O. keta*) from the Fraser River estuary were studied to determine whether their behaviour changed in response to predation risk and hunger level. In aquaria with and without predators, the fish were given a choice of two habitats, open water with a high prey density and vegetation with a low prey density; these habitats have advantages of high energy intake and protection from predators, respectively. Pink salmon were found more frequently in the vegetation when predators were present than otherwise. When hungry, however, pink salmon occupied the open area to a greater extent than when satiated. Chum salmon did not show as much difference in habitat choice as pink. In the presence of predators, satiated pink salmon ate less than hungry ones and also less than the fish without predators. Chum salmon did not show any differences in food intake in the different treatments. Thus, pink salmon responded to predation risk to a greater extent than did chum, in terms of habitat choice and food intake, and demonstrated a trade-off between predator avoidance and foraging.

L'auteur a étudié le choix de l'habitat et les activités de recherche de nourriture de juvéniles de saumon rose (*Oncorhynchus gorbuscha*) et kéta (*O. keta*) de l'estuaire du fleuve Fraser dans le but de déterminer si leur comportement était modifié par les risques de prédation et le niveau d'appétit. Dans des aquariums avec ou sans prédateurs, les poissons pouvaient choisir entre deux habitats : une zone dégagée avec une forte densité de proies et une zone de végétation à faible densité de proies. Ces habitats présentaient, respectivement, les avantages d'une ingestion énergétique élevée ou d'une protection contre les prédateurs. Les saumons roses se retrouvaient plus fréquemment dans la zone de végétation lorsque les prédateurs étaient présents. Mais lorsqu'ils avaient faim, ces saumons se trouvaient plus souvent dans la zone dégagée que lorsqu'ils avaient mangé. Les saumons kétas ne présentaient pas un écart aussi important quant au choix de l'habitat. En présence de prédateurs, les saumons roses rassasiés s'alimentaient moins que ceux qui avaient faim et moins que les poissons en l'absence de prédateurs. Les saumons kétas ne présentaient pas d'écarts d'ingestion alimentaire en fonction des régimes. Les saumons roses réagissaient donc plus que les saumons kétas au risque de prédation, ceci quant au choix de l'habitat et à l'ingestion de nourriture, et présentaient un certain équilibre entre l'évitement des prédateurs et la recherche de nourriture.

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The impact of predation risk on the feeding strategy of animals has recently received increased attention. The first optimal foraging theories were based on the assumption that an animal, in all situations, would try to maximize its energy intake to increase its fitness (Pyke et al. 1977). However, more recently, researchers have recognized that animals may make a trade-off between predator avoidance and maximum energy intake (e.g. Milinski and Heller 1978; Sih 1980; Cerri and Fraser 1983; Dill 1983; Werner et al. 1983; Power 1984). One choice for a forager could be between habitats with high food abundance and habitats offering protection from predators (Werner et al. 1983; Power 1984); the animal may choose the latter at the cost of reduced food intake. Another option would be to decrease foraging activity to reduce the suceptibility to predation (Dill and Fraser 1984; Magnhagen 1986). Few attempts have been made to incorporate predation risk in foraging models because of the problem with noncomparable units of measurements (Dill 1983; Wilzbach 1985). However, Mangel and Clark (1986) and McNamara and Houston (1986) have included alternative behaviours such as foraging, predator avoidance, and reproduction in comprehensive models. In both these models the metabolic state of an animal (e.g. energy level or gut contents) is the factor that decides the choice of actions made by the animal. This factor determines the animal's probability of survival, given a certain behaviour, and hence its reproductive success. Of the studies mentioned above, Dill and Fraser (1984) and Milinski and Heller (1978) looked at different hunger levels of the forager. In both these studies it was found that the fish took higher risks in terms of predation when they were hungry, as predicted by Mangel and Clark (1986) and McNamara and Houston (1986).

Juvenile salmon stay in estuaries for a period of time ranging from days to months, depending on species (Levy et al. 1979; Simenstad et al. 1982). It is believed that they use these areas

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for foraging, physiological transition from fresh to salt water, and refugia from predators (Simenstad et al. 1982). Even though there are high densities of potential predators in estuaries, juvenile salmon are found only occasionally in their stomachs (Simenstad et al. 1982; Jones 1986). This low predation rate may have several explanations. First, high turbidity in estuaries may decrease the visual field of predators (Gradall and Swenson 1982; Simenstad et al. 1982), and second, juvenile salmon may respond to high predation risk by choosing certain habitats or behaving in some other way that minimizes their exposure to predators (Simenstad et al. 1982).

In this study I used pink (*Oncorhynchus gorbuscha*) and chum salmon (*O. keta*) fry in aquarium experiments to investigate how their foraging behaviour may change with predation risk and hunger. The fish were tested in the presence and absence of predators and were given a choice of habitats: open water with a high prey density or vegetation with low prey density. These two habitats provide opportunities for high energy intake or protection from predators, respectively. I predicted that the fish would choose the two habitats differentially, depending on predation risk and satiation level, and hence show a trade-off between the demands for effective foraging and predator avoidance.

Methods

Pink and chum salmon fry were collected from Ladner marsh (49°6′N, 123°8′W) in the Fraser River estuary, British Columbia, Canada, by using a large seine as an intertidal trap to capture fish utilizing tidal channels (Levy and Northcote 1982). The fish were kept in storage tanks at least 1 wk before being used in experiments and were fed once a day with commercial fish food. The average fork length ($\bar{x}$ (SD)) was 36.5 mm (1.7) for pink and 41.6 mm (2.8) for chum.

Two oval-shaped "stream-tanks" were used for the experiments (Fig. 1). They were made with double Plexiglas walls, which created two sections, an outer and an inner. The inner and outer sections held approximately 125 and 200 L, respectively. The outer sections, containing the salmon fry, were divided in two equal-sized parts for the different treatments. Water flowed continuously into and out of the tanks, on both sides, creating a slow current. Water temperature was 10–13°C.

Three chinook salmon (*Oncorhynchus tshawytscha*) smolts, 15–20 cm long, were held in the inner section of one aquarium to simulate predation risk. These fish were clearly seen by the fry in the outer section of the aquarium, and the chinook occasionally tried to attack the fry. The other aquarium served as a control without predators.

In both tanks, the two halves in the outer section were divided into two different habitats. Half of the area was vegetated, i.e. plastic aquatic plants (type *Vallisneria spiralis*) were evenly distributed on the bottom to provide the fish with the opportunity to hide. The other half was open (Fig. 1). The plants were placed in the curved ends of the aquaria for two reasons. First, to minimize risk of prey disappearing through the outflow, they had to be released as far away from the outflow as possible; this meant releasing prey in the straight, open sides of the aquaria, while restricting plants to the ends. Second, the walls dividing the two parts of the outer section at the ends may have appeared to offer protection to chum and pink salmon, and therefore it was reasonable to associate the additional protection of the plants with these walls.

The fry were acclimated to the aquaria for 1 d before the experiment. Ten fish were put into each outer compartment, and a wooden enclosure (90 × 40 × 20 cm) was put around the predators in the inner section so that the fry would not see them.

To accustom the fish to a higher food density in the open habitat compared with the vegetation, they were fed live brine shrimp (*Artemia salina*) (2–10 mm long) once an hour, between 1030 and 1630 the day before the experiment, from a funnel in the middle of the open area. Furthermore, to create two hunger levels in the fish (for simplicity called satiated and hungry fish), the food items were supplied in two different amounts in the two halves of each aquarium (approximately 10 or 5 mg dry weight per feeding occasion). The satiated fish were also fed once in the morning, 4 h before the experiments started, with the same food amount as before, i.e. 10 mg. These methods resulted in four comparable treatments; satiated fish with

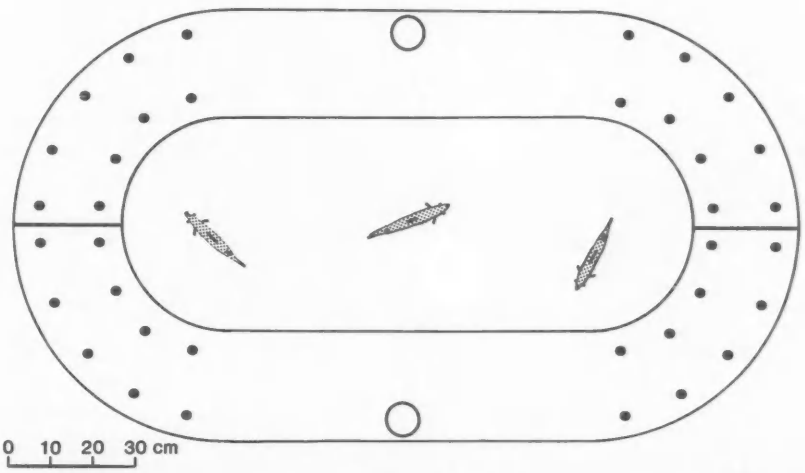

FIG. 1. Design of experimental aquaria, shown from above. Chinook "predators" are shown in the centre section, black dots show plant distribution, and open circles show the location of feeding funnels. Water depth was 25 cm.

TABLE 1. Two-way ANOVA on the proportion of fry in the open habitat; an arcsin $\sqrt{x}$-transformation was used.

		Pink			Chum		
Source	df	ss	F	p	ss	F	p
Predation risk	1	709.9	31.3	<0.001	80.6	0.4	n.s.
Hunger level	1	166.5	7.3	<0.05	16.6	0.1	n.s.
Predation risk × hunger level	1	14.7	0.6	n.s.	197.6	0.9	n.s.
Error	8	181.4			1740.9		

predators present and absent and hungry fish with predators present and absent.

Thirty minutes before the experimental feeding started the wooden enclosure around the predators was removed in the "predation risk" aquarium to expose the chinook smolts to the fry. The fish in all the compartments were fed, through the funnels, 20 *Daphnia* every 15 min for 2 h. The prey species was switched so that the prey consumed during the experiments could be distinguished in the stomachs from the prey taken earlier. The water current in the aquaria was sufficiently slow that the risk of *Daphnia* drifting all the way to the outflow during the experiment was small. The location of the fish (i.e. in the open area versus in the vegetation) was noted before each feeding (eight times per experiment). After 2 h the fish were removed, anaesthetized in MS222, and preserved in 4% formalin. The number of *Daphnia* in the stomachs was counted. The experiments were repeated three times for each species.

Results

Fish Location

For each experiment, the eight observations on fish location were pooled and analyzed by two-way ANOVAs using the arcsin $\sqrt{x}$-transformed proportions of fish found in the open habitat (Table 1). Pink salmon showed clear differences in location between different treatments (Fig. 2a). In the presence of predators, fewer fish occupied the open habitat compared with the control fish ($p < 0.001$; Table 1). Also, satiated fish were found less frequently in the open than hungry fish ($p < 0.05$; Table 1), independent of the presence or absence of predators.

Chum salmon did not demonstrate the same pattern as pink (Fig. 2b; Table 1). There were smaller differences between the chum treatments, and a higher variation within treatments, especially in the absence of predators.

There were no indications that the habitat choice of the fish changed with time in any of the species (Spearman rank test, n.s.), i.e. they did not become accustomed to the predators or learn that they were harmless during the experiment.

Feeding

Both predation risk and hunger level influenced the food intake in pink salmon (two-way ANOVA; Table 2). Satiated fish in the presence of predators had a lower prey consumption than fish in the other treatments (Table 3). In chum salmon, on the contrary, no significant differences in food intake were found between treatments.

Discussion

Pink salmon chose the two habitats in the aquarium experiment as expected, according to both predation risk and satiation level. In the presence of predators, the pink fry were found

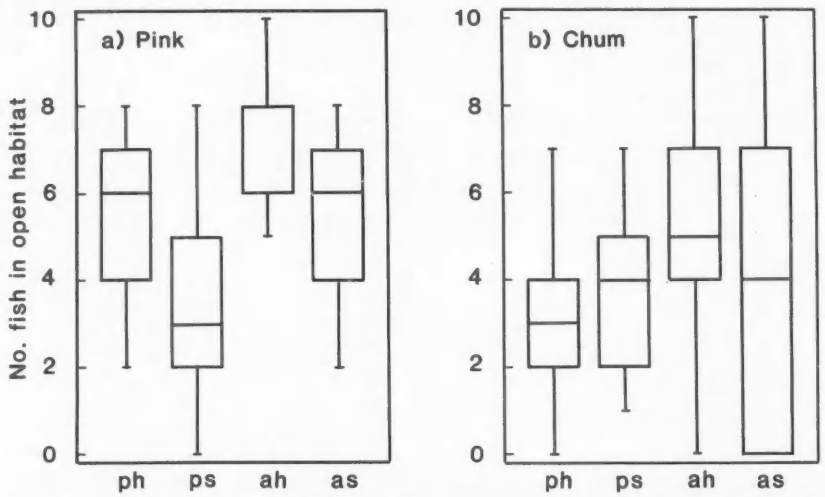

FIG. 2. Number of fish (of 10 possible) found in the open habitat in the experimental aquaria. The boxplots show the median, the upper and lower quartiles of the data, and adjacent values (= upper and lower quartile ± 1.5 × interquartile range) (p = predators present, a = predators absent, h = hungry fish, s = satiated fish; median for pink:ah = upper quartile = 8).

TABLE 2. Two-way ANOVA on the number of *Daphnia* eaten per fish in the aquarium experiments; a log-transformation was used.

Source	Pink				Chum			
	df	ss	*F*	*p*	df	ss	*F*	*p*
Predation risk	1	1.1	4.0	<0.05	1	0.04	0.1	n.s.
Hunger level	1	2.0	6.9	<0.01	1	0.01	0.04	n.s.
Predation risk × hunger level	1	0.4	1.4	n.s.	1	0.01	0.02	n.s.
Error	107	30.4			106	35.3		

TABLE 3. Mean number of *Daphnia* eaten per fish for different treatments in the aquarium experiments (n = number of stomachs analyzed).

	Pink		Chum	
	$\bar{x}$ (SD) range	*n*	$\bar{x}$ (SD) range	*n*
Predator present				
Hungry	8.8 (12.7) 0–48	24	9.1 (16.6) 0–78	28
Satiated	3.1 (7.1) 0–26	29	8.9 (17.3) 0–73	28
Predator absent				
Hungry	9.6 (13.1) 0–57	28	8.8 (14.3) 0–56	29
Satiated	8.8 (16.2) 0–79	30	8.0 (13.6) 0–62	24

more frequently in the vegetation, in comparison with those at the same satiation level in the control aquarium without predators. Hungry fish, however, were found to a greater extent in the open habitat with higher food availability than satiated ones. Thus, hungry pink salmon were taking greater risks to obtain food. As a consequence, the satiated fish in the predation risk aquarium ate less than the hungry fish, but also less than the satiated fish in the control. These fish demonstrate a trade-off between predation risk and energy intake, and the choice of action depends on the satiation level, i.e. the state of the fish, as predicted by Mangel and Clark (1986) and McNamara and Houston (1986).

Chum salmon responded less to the presence of predators than did pink, showing smaller differences in habitat choice in the absence and presence of predators. Furthermore, no differences were found in the habitat choice of chum with different satiation levels. The behaviour of chum salmon was more variable than that of pink. However, the pattern of behaviour in the two species appeared different. This makes it unlikely that significant differences in behaviour patterns of chum, similar to those found in pink salmon, could have been discovered simply by increasing the sample size in an effort to overcome the masking effects of variation in the chum data.

In the estuary, juvenile pink and chum salmon school in shallow eelgrass (*Zostera marina*) habitats during the day and disperse by night, a behaviour that may minimize exposure to predators (Simenstad et al. 1982; Healey 1982). Structural complexity decreases predatory success, either by reducing visibility or by modifying predator or prey behaviour (Savino and Stein 1982). In another study, juvenile bluegill sunfish (*Lepomis macrochirus*) were found to reside in vegetated areas when predators were present, even though the open habitat was more profitable with respect to food (Werner et al. 1983). The predation risk in this case was size specific and the fish left the vegetation and went into open habitats as they grew larger. Similarly, several species of armored catfish (Loricariidae) possess size-specific depth distribution patterns (Power 1984). The larger fish are more vulnerable to predation by birds than smaller ones and are found in deeper areas, where food is less abundant but predation risk is less severe than in shallow water.

Evidence for significant predation on salmon fry in estuaries is lacking (Simenstad et al. 1982). However, during the first weeks or months after entering the ocean, mortality of juvenile salmon is high, probably due to predation, primarily by coho salmon (*Oncorhynchus kisutch*) smolts (Parker 1971; Hargreaves and LeBrasseur 1985). The predation seems to be either size selective for smaller fish (Parker 1971) or species selective for pink salmon (Hargreaves and LeBrasseur 1985). Thus, it may be more important for pink salmon than for chum to adjust their behaviour to the risk of predation, which they actually did in my experiments. However, more studies need to be performed on costs and benefits of different behaviours to clarify their significance for the survival and fitness of the animal.

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Hydroacoustic Signal Classification of Fish Schools by Species[1,2]

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Rose, G. A., and W. C. Leggett. 1988. Hydroacoustic signal classification of fish schools by species. Can. J. Fish. Aquat. Sci. 45: 597–604.

Features extracted from hydroacoustic backscatter from fish schools enabled classification by species. Target species were cod (*Gadus morhua*), capelin (*Mallotus villosus*), and mackerel (*Scomber scombrus*), observed in the northern Gulf of St. Lawrence during 1985–86. Two features of internal school density (20 log *R* amplification) were the best discriminators. These were mean standardized peak to trough distance (SPT) and mean distance between voltage peaks (PP). Quadratic discriminant functions based on the variables SPT, PP, an inverse coefficient of variation, school depth, and off-bottom distance correctly classified 93% of schools (1986). These functions also correctly classified 93% of cod and capelin schools acoustically sampled independently during 1985. The target strength of individual fish was a less successful discriminator of species. For cod and capelin of known length, average target strength (TS) was a linear function of length: TS (decibels) = $-65 + 20 \log_{10}$ length (centimetres). Mackerel had target strengths that were 8–12 dB less than those of cod of equivalent length. Quadratic discriminant functions based on target strength, school depth, and off-bottom distance correctly classified 77% of schools by species. Our methods are generalized to any schooling species or environment.

Certaines caractéristiques des échos hydroacoustiques en provenance de bancs de poissons ont permis d'en préciser l'espèce. Les espèces visées, la morue (*Gadus morhua*), le capelan (*Mallotus villosus*) et le maquereau (*Scomber scombrus*), avaient été étudiées dans la partie nord du golfe du Saint-Laurent en 1985–1986. Les meilleurs discriminateurs étaient représentés par deux caractéristiques de la densité interne des bancs (amplification de 20 log *R*). Il s'agissait de la distance moyenne normalisée entre les pics et les creux (PCN) et la distance moyenne entre les pics de tension (PT). Des fonctions de discriminants quadratiques basées sur les variables PCN, PT, un coefficient inverse de variation, la profondeur du banc et la distance à partir du fond, ont permis de déterminer correctement l'espèce de 93 % des bancs (1986). Ces fonctions ont aussi permis de déterminer correctement à 93 % l'espèce de bancs de morue et de capelan ayant fait l'objet d'un relevé acoustique indépendant en 1985. La force de l'écho des poissons individuels s'est avérée être un discriminant de l'espèce moins efficace. Dans le cas de morues et de capelans de longueur connue, la force moyenne de l'écho (FE) était une fonction linéaire de la longueur : FE (decibels) = $-65 + 20 \log_{10}$ longueur (centimètres). La force de l'écho réfléchie par les maquereaux était inférieure de 8 à 12 dB à celle des morues de longueur équivalente. Les fonctions à discriminants quadratiques basées sur la force de l'écho, la profondeur du banc et la distance du fond ont permis de déterminer correctement à 77 % l'espèce de poisson des bancs. Les auteurs ont généralisé leurs méthodes à l'ensemble des espèces formant des bancs et des environnements.

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The application of hydroacoustic assessment techniques has been limited by an inability to objectively discriminate among taxonomic groups of sound scatterers (Thorne 1983). To date, most workers have used identification methods based on net sampling near the acoustic transect and on visual interpretation of echograms. In many instances, however, neither of these techniques has enabled objective taxonomic discrimination.

Taxonomic compositions of acoustically determined biomasses are often assigned by reference to the composition of net samples taken near the acoustic transect. There are two central problems with this approach. First, net samples seldom reflect actual species or size composition; second, net samples in practice cannot achieve spatial or temporal sampling which is comparable with that of acoustic sampling (R. E. Thorne. "Hydroacoustics and ground truth." ICES/FAO International Symposium on Hydroacoustics, Seattle, WA, June 22–26, 1987). In single-species environments or where schools are large, widely separated, and have a high catchability, these biases of net sampling may be small. In multispecies environments, especially where schools are small, interspersed, and have a low or varying catchability, net sampling is often unworkable even as a rough means of taxonomic identification.

Visual interpretations of echograms, with taxonomic discrimination based on "rules of thumb," represent the first attempts to use the acoustic signal itself to identify targets by taxa. Such methods are used by many commercial fishermen. The "rules of thumb" pertain to multivariate assessments of voltage strength (evident as shades of grey on an echogram)

[1]Contribution to the program of GIROQ (Groupe Interuniversitaire de Recherche Océanographique du Québec).

[2]A similar paper entitled "Classification of acoustic images of fish schools by species" was presented to the ICES/FAO International Symposium on Fisheries Acoustics, Seattle, WA, June 22–26, 1987.

and school shape, size, and position in the water column, generally mediated by knowledge of the species likely to be encountered. These techniques suffer from subjectivity and are labor intensive.

Several attempts have been made to quantify, make more objective, and automate echogram interpretation by measuring various characteristics and using these as input to pattern recognition algorithms (Azzali 1982; Nion and Castaldo 1982). These techniques, although an improvement over subjective visual estimates, have a major weakness. Echograms are simply rough graphic representations of amplified acoustic backscattered energy and present a smoothed picture of any higher frequency information contained in the signal. Echogram interpretation therefore is inherently wasteful of information that may assist taxonomic classification.

Several recent approaches use information present in the digitized backscattered signal itself to classify targets. These approaches are of three major types: (1) dual-beam and split-beam techniques for sizing sound scatterers, (2) wideband multifrequency response techniques, and (3) feature extraction from narrowbeam echosounder signals.

Dual-beam and split-beam techniques have shown potential for separating organisms of different sizes (Traynor and Ehrenberg 1979; Dickie et al. 1983; Burczynski and Johnson 1986; Foote et al. 1986). These techniques are constrained, however, by their power to resolve single targets. Many species school at densities sufficiently high to make resolution of single targets problematic (Dickie et al. 1983). An additional limitation is that size classification enables taxonomic classification only when target taxa have discrete size distributions.

Other workers have used complex wideband and multiple-frequency echosounders to characterize responses of targets of various sizes and taxa to a range of frequencies. These techniques are based on the theoretical frequency dependence of the relationship between target size and backscattering strength (Holliday 1972). Multifrequency response techniques have been used in attempts to separate fish from plankton (Saetersdal et al. 1984) and to classify fish by species (Zakharia and Sessarego 1982; E. J. Simmonds and F. Armstrong. "A wide band echosounder: measurements on cod, saithe, herring, and mackerel from 27 to 54 kHz." ICES/FAO International Symposium on Fisheries Acoustics, Seattle, WA, June 22–26, 1987).

A technically simpler empirical approach employing narrowband echosounders has been to characterize and then recognize the patterns of backscatter from organisms or groups of organisms of specific types (Deuser et al. 1979; Giryn et al. 1979; D. Vray, G. Gimenez, and R. Person. "Attempt of classification of echosounder signals based on the linear discriminant function of Fisher." ICES/FAO International Symposium on Fisheries Acoustics, Seattle, WA, June 22–26, 1987). These authors used variations of Fourier transformations of the signal to classify targets. To date, however, these potentially powerful approaches to signal classification and recognition have yielded limited success.

In our approach to target identification, we hypothesized that the acoustic signal from schools of fish should contain information sufficient to enable discrimination by species. Two types of characterization were investigated: (1) target strengths of individual fish and (2) features of the backscattered energy and density structure of whole schools. We tested these concepts using acoustic signals from free-ranging schools of cod (*Gadus morhua*), capelin (*Mallotus villosus*), and mackerel (*Scomber scombrus*).

Target strength is length dependent in fishes with swim bladders (Midttun 1984). In our study these are represented by cod and capelin. Fishes without swim bladders, such as mackerel, have target strengths well below those of much smaller fishes with swim bladders (Nakken and Olsen 1977; Foote 1980; Edwards and Armstrong 1983). We thus hypothesized that target strengths of individual fish from schools of cod would be greater than those from schools of capelin, which in turn would be greater than those from schools of mackerel.

The acoustic backscattered energy from a fish school, after correction for spreading and attenuation losses (amplification $20 \log R + 2\alpha R$; where R = range and α = attenuation coefficient of sound in water), is proportional to the density of the school (MacLennan and Forbes 1984). An acoustic backscatter profile can thus be made analogous to a vertical section of school density. We hypothesized that three types of characteristics of the school density profile would be species dependent and thus allow classification: (1) position in water column, (2) external shape and size, and (3) internal density structure.

In this paper, we present the results of these investigations, develop specific acoustic criteria for discriminating among schools of cod, capelin, and mackerel, and discuss the general implications of our findings to the taxonomic classification of acoustic signals.

Methods

This study was carried out at Brador Bay, Quebec, Canada, on the north shore of the Gulf of St. Lawrence. Dockside measurements were made at an abandoned deepwater schooner wharf at Bassin Ile within Brador Bay.

To establish a fish size–target strength relationship, immobilized cod and mackerel and free-swimming capelin of known length were ensonified at dockside. Cod and mackerel were caught by hand line in shallow water (< 25 m). Fish that were obviously injured or overly traumatized were released. Healthy fish were transported to dockside in a live box. Unfortunately, mackerel expired quickly. At dockside the transducer was fixed over 10 m of water. Twenty five cod of fork lengths 27–122 cm and five mackerel of fork lengths 36–43 cm were measured for target strength. Fish were suspended at a distance of 4–8 m from the transducer, in its far field, from monofilament line tied through punctures made in the dorsal fin and behind the head. Mackerel required the attachment of a weighted line to their ventral surface to keep them in dorsal aspect. SCUBA divers and line handlers at the surface attempted to keep the whole fish within the beam, with their long axis parallel to the transducer face, during measurement. As the basal diameter of the half-power ensonified cone of the narrow beam ranged from approximately 0.7 m at 4 m from the transducer to 1.4 m at 8 m from the transducer, the larger cod just fit into the beam. Cod were docile during this procedure; mackerel were dead when measured. Several hundred pings were recorded from each fish. On four occasions during the above work, capelin schools were ensonified in dorsal aspect as they swam freely beneath the transducer. The lengths of these fish were determined visually at close range by SCUBA divers.

We used a Biosonics model 105 echosounder (120 kHz), fitted with a dual-beam transducer (10 and 25°) mounted in a V-fin towing body, to make all acoustic measurements. Pulses of 0.8 ms duration were generated at a rate of $5 \cdot s^{-1}$. A Biosonics tape recorder interface and Sony video recording system stored all data for analysis. When working at sea, the transducer was

towed beside the 9-m vessel at a depth of approximately 2 m. Transducer stability was enhanced by a series of movement-damping springs which isolated the movements of the boat from that of the towed body. Boat speed was approximately 10 km·h^{-1}. Under these conditions, noise peaks at 80 m of depth were approximately 20 mV with 20 log R amplification and 50 and 100 mV on the narrow and wide beams, respectively, with 40 log R amplification. Field calibration carried out on a 5.08-cm-diameter stainless steel ball bearing indicated that its target strength was a stationary time series (constant mean and variance) with SE of 0.3 dB.

A total of 46 schools of capelin, 70 schools of cod, and 11 schools of mackerel were positively identified during the summers of 1985 and 1986. Cod and mackerel schools were considered positively identified if, after acoustic sampling, a multihooked jig line lowered into the school hooked a single species on three successive attempts. Jig lines were guided into the school by reference to the echogram. Capelin schools were considered positively identified when observed visually from the surface or by SCUBA divers. Capelin identified from the surface were also subjected to the jig line test, and in all cases, nothing was hooked. Attempts were made to sample schools of varying sizes and at varying depths from the surface to approximately 50 m. A few cod schools were positively identified at greater depths. During 1985, acoustic data were recorded from only the narrow beam (thus, no target strengths are reported). Data collected during 1986 were integrated in real time with a Biosonics model 121 digital echo integrator. In 1986, both narrow- and wide-beam signals were also recorded on tape with 40 log R amplification, to be subjected later to target strength analysis.

Target strengths were determined with a Biosonics model 181 dual-beam processor following methods given by Traynor and Ehrenberg (1979). Echoes were classified as single fish if the peak signal on the narrow beam was greater than 100 mV and half height (−6 dB) width was between 0.55 and 1.1 ms. A −18 dB echo width criteria was not used because its use reduced sample size considerably. Target strengths calculated using the −6 dB criterion and those calculated using both the −6 dB criterion and a −18 dB maximum width of 2 ms were significantly correlated ($r = 0.80$). The slope of this relationship (0.88) was not significantly different from 1 ($P > 0.05$).

To analyze school characteristics, voltages made proportional to density ($V^2 \propto$ density; 20 log R amplification (Burczynski 1982)) were digitized at a frequency of 16.5 kHz on a TECMAR Labmaster A/D converter and then stored on computer disc. For each school, two randomly selected sequences of four or five consecutive echosounder pings were recorded. Schools were delimited by the occurrences of voltages of less than 100 mV for a duration of 3.0 ms (2.25 m). A computer program was used to calculate the following criteria for each sample: (1) off-bottom distance (metres), (2) depth of school (metres), (3) mean squared voltage, (4) standard deviation of squared voltage, (5) maximum squared voltage, (6) mean distance between within school voltage peaks (metres) (hereafter called PP), and (7) mean peak to trough squared voltage standardized to the mean squared voltage (hereafter called SPT) (Fig. 1). Peaks and troughs were defined as having higher and lower voltages, respectively, than the two preceding and two following voltages.

Discriminant analysis was used to characterize and classify schools by species. To determine the variables to be entered into the discriminant analyses, variables identified in the preceding paragraph (20 log R) were subjected to stepwise selection. From the 40 log R signal, the following variables were entered: target strength (TS), school depth, and off-bottom distance. We attempted to use variables that were robust against variability in amplifier gain settings and system calibration. Thus, an inverse coefficient of variation (mean/standard deviation) (hereafter called CV) was used instead of standard deviation. Prior to entry into discriminant analyses, variables whose distributions suggested non-normality (PP, school depth, and off-bottom distance) were subject to $\log_e + 1$ transformation (Shapiro–Wilk statistic, $P > 0.01$). Covariance matrices were tested for homogeneity (Morrison 1976). If a chi-square test indicated that the covariance matrices of the three species were not homogeneous, the use of a pooled covariance matrix and linear discriminant functions was rejected in favor of quadratic discriminant functions based on within-group matrices (Morrison 1976). Quadratic discriminant functions can be expressed as

$$f(x_1,\ldots,x_n) = a_{11}x_1^2 + \ldots + a_{nn}x_n^2 + 2a_{12}x_1x_2 + \ldots + 2a_{1n}x_1x_n + \ldots + 2a_{n-1,n}x_{n-1}x_n$$

where $x_1,\ldots,x_n$ are the values of the measured criteria and $a_{11},\ldots,a_{nn}$ are the coefficients of the quadratic function. Discriminant analyses were carried out on the programs of SAS and SAS/STAT for personal computers (SAS Institute Inc. 1985). Differences were considered significant if $P < 0.05$ unless stated otherwise.

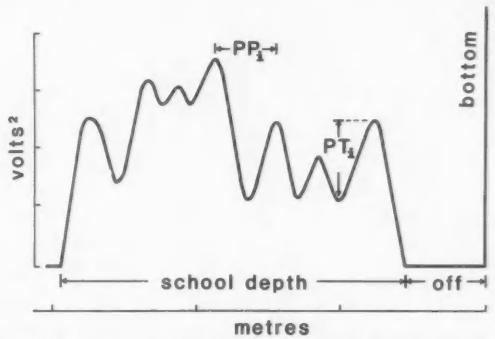

FIG. 1. Measurement of school descriptors. Time measures (school depth, off-bottom distance, PP) calculated in m from initial measure (ms). $PP = \sum_1^n PT_i \cdot n^{-1}$). Voltage measures (maximum, mean, SD, and peak to trough distance (PT) in V^2. SPT is the unitless quantity $SPT = \sum_1^n PT_i \cdot n^{-1} \cdot (\overline{V^{-2}})$.

Results

Target Strength

The mean target strengths of cod measured at dockside showed a positive linear relationship to fish length (TS (decibels) = $-67.5 + 21 \log_{10}$ length (centimetres) ($n = 25$, $r = 0.95$, SE = 1.0 dB) (Fig. 2). Capelin schools ensonified at dockside had a mean length of 16.5 cm and a mean target strength of −41.0 dB (SD = 1.19) (Fig. 2). The cod length – target strength regression, extrapolated to 16.5 cm, predicted a mean target strength of −41.9, which did not differ significantly from the value measured for capelin.

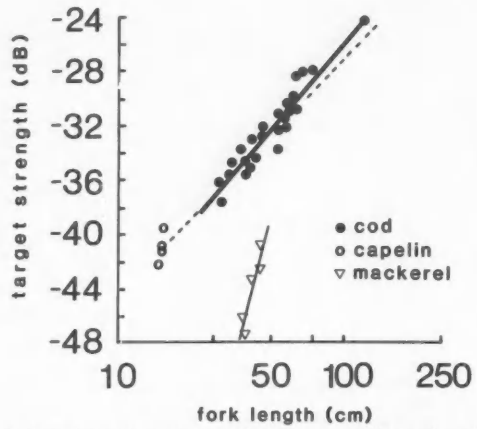

FIG. 2. Dorsal aspect target strengths of cod, capelin, and mackerel measured at dockside at Brador Bay, Canada, during 1986. Cod and mackerel were tethered during measurement; capelin were free swimming. Cod and capelin together yield the regression TS(dB) = $-65 + 20 \log_{10}$ length (cm) ($n = 29$, $r = 0.97$, SE = 1.0 dB). Mackerel yield the regression TS(dB) = $-151 + 67 \log_{10}$ length (cm) ($n = 5$, $r = 0.92$, SE = 1.0 dB).

The mean target strength of mackerel was also a linear function of the length of the fish: TS(decibels) = $-151.3 + 67 \log_{10}$ length (centimetres) ($n = 5$, $r = 0.92$, SE = 1.0 dB). This relationship was orders of magnitude different from the equivalent regression for cod. Mackerel target strength averaged -44.0 dB and was 8–12 dB less than the target strength of cod of equivalent length. Mackerel with lengths of approximately 40 cm had target strengths similar to those of 16.5-cm capelin (approximately -42 dB).

Mean target strengths measured at sea were -31.4 dB for cod ($n = 1576$), -41.8 dB for capelin ($n = 1681$), and -42.7 dB for mackerel ($n = 42$) (Fig. 3). Capelin and mackerel target strengths were normally distributed around their means. Cod target strength had a bimodal distribution, with modes at -26 and -50 dB. The mean target strength of cod was greater than the mean target strengths of capelin and mackerel (Mann–Whitney test, $P < 0.05$). Capelin and mackerel target strengths did not differ (Mann–Whitney test, $P > 0.05$). All three species exhibited a wide range of target strength.

Target strengths were determined for 55 of 60 cod, capelin, and mackerel schools measured at sea (Fig. 4). No single targets could be resolved from the five schools (four mackerel and one capelin) for which no target strength is reported. For many schools the number of single targets assessed was low (in six cases, $n = 2$). The modal frequencies of the mean target strength of cod and capelin schools were 14 dB apart. The mean target strength of the seven mackerel schools ranged from -30 to -48 dB, with a modal frequency similar to that of capelin.

Stepwise discriminant analysis selected all three variables entered as discriminators of species in the order target strength, school depth, and off-bottom distance. Quadratic discriminant functions were calculated because covariance matrices were heterogenous ($X^2 = 103$, $df = 12$, $P < 0.001$) (Table 1). These functions correctly classified 77% of schools measured during 1986 (or 84% if only those schools with estimated target strength are included). Classification success was unequal among species. For cod, classification was 100% correct

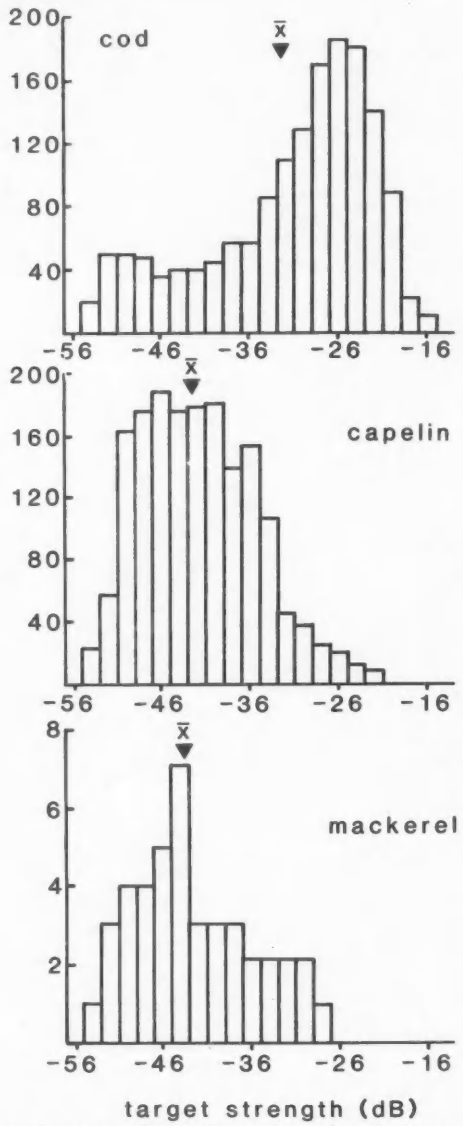

FIG. 3. Target strengths of cod, capelin, and mackerel measured at sea at Brador Bay, Canada, during 1986. Mean ± SD (n): cod, -31.4 ± 9.4 (1576); capelin, -41.8 ± 6.6 (1681); mackerel, -42.7 ± 6.8 (42).

whereas for capelin and mackerel, only 61 and 55% of schools, respectively, were classified correctly.

Density Signal

The PP and SPT densities both differed significantly among the three species (Table 2). Vertical density profiles of capelin schools were characterized by low values of SPT and PP (Fig. 5). By contrast, mackerel schools had high values of SPT and intermediate values of PP. Cod schools were characterized by intermediate values of SPT and high values of PP. No other measured criteria differed significantly among all three species, although capelin schools were greater than both cod and mack-

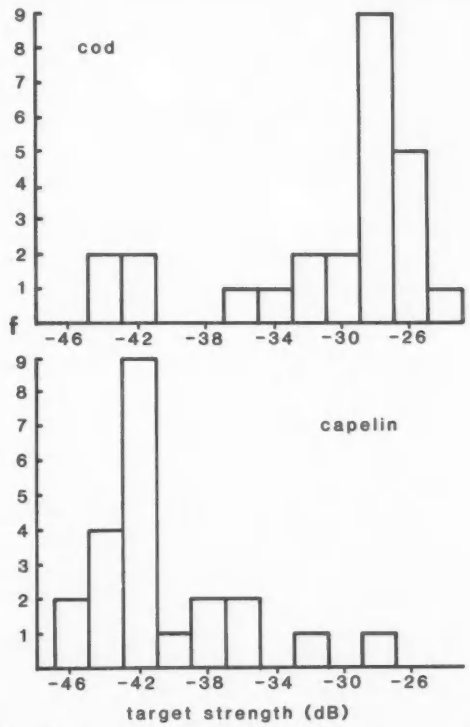

FIG. 4. Mean target strengths of schools of cod and capelin measured at sea at Brador Bay, Canada, during 1986.

TABLE 1. Discriminant analysis classification by target strength, school depth, and off-bottom distance of 23 capelin, 26 cod, and 11 mackerel schools measured in 1986. "No class" indicates schools for which all variables were not available (i.e. TS, $n = 0$). Percentages in parentheses.

	Classified into species				
From	Capelin	Cod	Mackerel	No class	Total
Capelin	14(61)	5(22)	3(13)	1(4)	23(38)
Cod	0(0)	26(100)	0(0)	0(0)	26(43)
Mackerel	0(0)	1(9)	6(55)	4(36)	11(18)
Total	14(23)	32(53)	9(15)	5(8)	60(100)

TABLE 2. Mean untransformed values of descriptors of capelin, cod, and mackerel schools measured during 1986 (n in parentheses). Underlined values form nonsignificantly different groups (Duncan's multiple range test, $P < 0.05$). For off bottom, school depth, and PP, statistical comparisons based on $\log_e + 1$ transformations.

	Cod(26)	Mackerel(11)	Capelin(23)
Off bottom (m)	0.52	1.96	4.60
School depth (m)	4.29	2.53	13.02
Maximum voltage (V^2)	2.7	0.8	20.0
Mean voltage (V^2)	0.58	0.11	2.30
SD of voltage (V^2)	0.72	0.19	4.16
PP (m)	0.87	0.65	0.75
SPT	2.04	3.51	1.55

erel in school depth and mean, maximum, and total relative density. Although mean off-bottom distance did not differ among species, cod tended to be associated most closely with the bottom and capelin were the most pelagic.

Stepwise discriminant analysis selected species discriminators in the following order: SPT, PP, school depth, CV, and off-bottom distance. Quadratic discriminant functions were calculated because covariance matrices were heterogenous ($X^2 = 186$, df $= 30$, $P < 0.001$) (Table 3). These functions classified correctly 93% of all schools measured in 1986 and from which the functions were derived (Table 4). All species were classified with good success (>90% correct).

The quadratic discriminant functions generated from the 1986 data correctly classified 93% (62 of 67) of cod and capelin schools ensonified during 1985 and for which the discriminating criteria were calculated (Table 5).

A stepwise discriminant analysis, which included all of the density signal variables indicated above plus target strength, selected target strength after SPT and PP, but removed target strength as a discriminator after the entry of the CV. The final selection of variables was the same as indicated above for the density signal.

Discussion

Target Strength

The fish length–target strength relationship reported here for cod is very similar to that published previously for cod at 120 kHz by Nakken and Olsen (1977). It is also in general agreement with summaries of length–target strength relationships for this species (Midttun 1984; Foote 1987). Mean target strength of capelin schools of known average length did not differ from that predicted by the regression of target strength on length for cod, suggesting that capelin and cod may be described by a single regression. Combining the data for cod and capelin yields the regression TS (decibels) $= -64.9 + 20 \log_{10}$ length (centimetres) ($n = 29$, $r = 0.97$, SE $= 1.0$ dB).

Our data support the existence of a linear relationship between length and mean target strength for mackerel, but suggest that this line is on average 10 dB lower, and has a steeper slope, than the regression for cod and capelin. These findings are consistent with previous comparisons of the target strengths of fishes having swim bladders with those that do not (Foote 1980; Edwards and Armstrong 1983). Nakken and Olsen (1977) did not report a length–target strength regression for mackerel, but target strengths reported for mackerel were 10–11 dB less than for cod of similar size. The mean mackerel target strength we determined at sea (−42.7 dB; SE = 1.0) did not differ significantly from that reported by Nakken and Olsen (1977) for mackerel of length 29–34 cm (−41.9 dB; SE = 1.0). The mean target strength relationship we developed for dead mackerel of known length, however, appears to predict somewhat lower values than reported by these authors.

The hypothesis that the mean target strength would differ among schools of cod, capelin, and mackerel, and thus allow classification of these species, was rejected. Our data suggest that there are two reasons for this result. First, as the measurements on fish of known length show, the target strengths of larger mackerel are similar to those of capelin; hence, target strength cannot be expected to be a good discriminator of these species. Second, schooling behavior may create fish densities at which the selection of single fish echoes becomes a highly

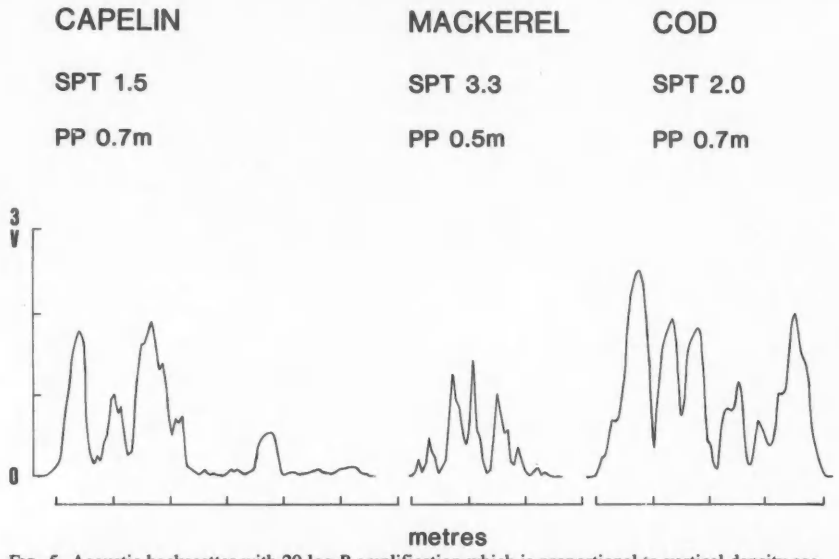

FIG. 5. Acoustic backscatter with 20 log R amplification which is proportional to vertical density sections through schools of capelin, mackerel, and cod of approximately equal maximum density. Signal patterns and values of SPT and PP indicated were typical of these species at Brador Bay during 1985 and 1986.

TABLE 3. Quadratic discriminant function coefficients generated from 23 capelin, 26 cod, and 11 mackerel schools measured during 1986. Variables are SPT, $\log_e$ (PP + 1) = PP, CV, $\log_e$ (school depth + 1) = SD, and $\log_e$ (off-bottom + 1) = OFF. Classification is to species with maximum value of D = constant + d_1 (SPT²) + ... + d_i (CV²) + ... + $2d_i$ (SPT) (PP) + ... + $2d_i$ (OFF) (CV). For details see Methods.

	Capelin	Cod	Mackerel
Constant	−350.489	−297.405	−185.910
SPT	−43.207	−8.189	−3.367
PP	−492.337	−146.540	−254.749
SD	−2.127	−19.786	−22.184
OFF	−0.790	−44.229	−7.911
CV	−54.055	−31.729	−150.949
SPT·PP	−17.508	−9.134	13.285
SPT·SD	−2.338	−8.063	−3.811
SPT·OFF	1.790	5.199	−2.033
SPT·CV	4.691	−3.705	−1.734
PP·SD	−14.666	−21.044	3.621
PP·OFF	0.780	3.692	20.428
PP·CV	20.198	−7.779	−54.933
SD·OFF	0.156	4.694	7.477
SD·CV	−3.427	−5.752	−6.317
OFF·CV	−2.194	−4.193	1.792

TABLE 4. Quadratic discriminant analysis classification, by SPT, PP, coefficient of variation, school depth, and off-bottom distance, of 23 capelin, 26 cod, and 11 mackerel schools measured in 1986. Details of discriminant functions given in Table 3. Percentages in parentheses.

	Classified into species				
From	Capelin	Cod	Mackerel	No class	Total
Capelin	21(91)	2(9)	0(0)	0(0)	23(38)
Cod	0(0)	25(96)	1(4)	0(0)	26(43)
Mackerel	1(9)	0(0)	10(91)	0(0)	11(19)
Total	22(37)	27(45)	11(18)	0(0)	60(100)

TABLE 5. Classification of 23 capelin and 44 cod schools measured in 1985 employing the quadratic classification functions generated from the 1986 data. Details of discriminant functions given in Table 3. Percentages in parentheses.

	Classified into species				
From	Capelin	Cod	Mackerel	No class	Total
Capelin	20(87)	3(13)	0(0)	0(0)	23(34)
Cod	2(5)	41(93)	1(2)	0(0)	44(66)
Total	22(33)	44(66)	1(1)	0(0)	67(100)

biased process. This is especially true for mackerel and capelin. In such cases, target strength appears to become an unpredictable quantity, dependent on unknown variations in packing density of the school, behavioral patterns, and the random occurrence of nonrepresentative single fish or echoes deemed to be single fish by the selection criteria. In addition, some misclassified single targets may contaminate the target strength data. This holds especially for cod, where the −50 dB mode would suggest, based on the fish length–target strength regression, the presence of 8-cm cod. We consider the presence of small cod with adult fish highly unlikely. This −50 dB mode is more likely either a function of an unknown behavioral variation or represents an unidentified organism. We conclude that species separations can be made employing mean target strengths, but only when target species have reasonably discrete target strength distributions (such as cod and capelin) and when the schooling structure of the targets allows the resolution of a reasonable number of single targets.

Classification by School Descriptors

Classification of cod, capelin, and mackerel schools by the school descriptors was highly successful. This success was

achieved largely because of consistent and predictable differences in SPT among species. PP also had strong discriminatory power. All stepwise selections chose SPT first and PP second an discriminators of species. We believe that these measures integrate differences in acoustic reflection and resonance of schools which differ in size of individuals, internal school structure, and packing density. PP was positively correlated with target strength ($r = 0.50, P < 0.05$). SPT was negatively correlated with mean school density ($r = -0.29, P < 0.05$).

It is important to note that SPT and PP values must be interpreted in relation to the 0.8-ms pulse width (59 cm) used in this work. Density layers of acoustic targets separated by less than this distance could not be fully resolved. In addition, the curved base of the acoustic beam will further decrease the sampling resolution of horizontal layers of fish. This decrease in resolution will increase with depth as a result of the increasing width of the beam with range. For example, at 50 m, assuming that fish layers are normal to the acoustic axis, the effective vertical resolution increases to 79 cm. Thus, all but the larger cod may at times have schooled at densities too great for complete vertical resolution of single layers of fish. Despite this limitation, density layers separated by less than the pulse width may be partially resolved. The degree of partial resolution is thought to be the basis of the observed differences in SPT and PP.

We believe that the SPT and PP measures reflect internal school structure. Nearest neighbor distance (NND) has often been used as a criterion of internal school structure. Pitcher and Partridge (1979) showed that under laboratory conditions, NND averaged near 1 body length. A recent laboratory study of schooling structure under variable light conditions showed that mackerel maintain NND of 0.3–1.8 body length (Glass et al. 1986). In the present work, assuming icosahedral packing of fish with density layers normal to the acoustic axis, NND values were identical for mackerel and cod (3.2 mean body lengths, calculated from mean target strength of mackerel at sea applied to the regression of length on target strength). These NND values are higher than predicted by Pitcher and Partridge (1979), but considering the comparison involves field and laboratory data, the differences are not remarkable. Several other derivations of NND or volume occupied by each fish from field data showed values well above those predicted for cod, herring, and capelin (Radakov 1973; Serebrov 1976; Cushing 1977). The similarity of the theoretical values of NND and our empirically determined values supports the hypothesis that SPT and PP reflected actual nonrandom variations in school structure for cod and mackerel.

For capelin, PP probably did not represent actual vertical density spacing. Mean NND (10 body lengths) appeared to be inconsistent with that predicted by Pitcher and Partridge (1979). For capelin, resolution of peaks was probably aliased as the distance between density layers in relation to pulse width decreased. This interpretation is strengthened by the low value of SPT (1.5), which suggests that density layers were closely spaced. Use of a narrower pulse width would probably increase the discrimination power of PP.

The potential usefulness of the classification measures depends on their robustness against variations in acoustic system calibration and fish school parameters. We have attempted, by avoiding the use of unstandardized measures of voltage, to derive discriminating measures which are robust against variable system output. Potential variability in the fish school parameters, beyond that encountered here, and the effect of such variability on the success of the discriminant functions is more difficult to assess. The form, size, and density of fish schools are likely to vary seasonally and with changing environmental conditions (Radakov 1973). We caution that any attempt to use these discriminant functions outside the particular environment of this study will require a reassessment and recalibration of the quadratic coefficients. In particular for mackerel, which in inshore areas avoid boats and school at shallow depth, the values shown here may not be appropriate for deeper waters.

Classification in Other Systems

We have demonstrated a specific case of how signal feature extraction techniques can be applied to fisheries acoustics. The specific features found to be successful discriminators of species in the environment dealt with here, and the methods used to extract these features, should be regarded as being particular examples from a wide range of potential signal descriptors. In other systems, other methods and other features may provide good discrimination. For example, Fourier transformations can be employed to describe the wave forms of school and individual fish echoes (Holliday 1972; Deuser et al. 1979). We advocate that discriminant functions be developed on a case-by-case basis, employing an empirical approach, wherein the success of the classification is paramount. An ecological rationale will assist workers in identifying features likely to have discriminatory power.

Discriminant techniques are easily automated and well suited to taxonomic classification of acoustic signals (Fisher 1936). However, Fisher's technique was linear and required that the covariance matrices of the groups be homogeneous. We found that the covariance matrices of the school descriptors were heterogeneous, and we believe that this heterogeneity has a biological basis. For example, cod schools ensonified near the bottom were on average thicker than those found well off the bottom. Capelin and mackerel schools, by contrast, were thicker when located further off bottom. For all species, thicker schools were on average denser. Thus, cod schools near bottom were denser, but capelin and mackerel schools were denser when more pelagic. These differences are thought to reflect behavioral differences between cod, a species usually associated with the bottom, and capelin and mackerel, which are pelagic. Thus, heterogeneity of covariance matrices of these types of signal descriptors may be universal and require use of the quadratic approach.

In conclusion, we have shown that species classification of schooling fishes is possible using an array of discriminators extracted from the acoustic signal. These techniques are amenable to automated processing and in conjunction with integration and target strength assessment can provide data on fish abundance by species.

Acknowledgements

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Precision of Acoustic Fish Stock Estimates

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We derive efficient estimators of Q, the total fish quantity in an area sampled by acoustic survey, and the variance of Q. The estimators are valid when autocorrelation is insignificant, the nonzero samples have stationary statistics, and the distribution of the nonzero samples is normalised by a power transformation. Zero samples are treated separately, thus avoiding one source of nonstationarity. The Box–Cox test is applied to the nonzero data to determine the best λ to normalize the data through the transformation $Z = (x^\lambda - 1)/\lambda$. Estimation formulae are presented for $\lambda = 1/2, 1/3, 1/4, 1/6$ and the log transform $Z = \log x$ which corresponds to $\lambda = 0$. Our methods have been applied to data from the 1984 and 1985 North Sea herring surveys. The sampling error is close to 25% in both years. Both the time between shoal encounters and the shoal size can be described by three-parameter Weibull distributions. Theoretical expressions for the mean fish quantity, and the variance of this mean, are presented as functions of the Weibull parameters. Computer simulations based on these distributions confirm the validity of the proposed estimation procedure. The estimators suggested by the Box–Cox test have the least bias. Other estimators exhibit severe bias. In particular, if log transforms are used when the data are not log-normally distributed, and Box–Cox suggests $\lambda > 0$, the mean fish quantity and its variance are both likely to be overestimated.

Les auteurs ont obtenu des estimateurs efficaces de Q, la quantité totale de poisson dans une zone inventoriée par relevé acoustique, et de la variance de Q. Ces estimateurs sont valables lorsque l'autocorrélation est pratiquement nulle, que les échantillons autres que 0 présentent des caractéristiques statistiques stationnaires et que leur distribution est normalisée par une transformation de puissance. Les échantillons 0 sont traités de façon distincte, ce qui permet d'éliminer une source de non-stationnarité. Le test de Box–Cox est appliqué aux données autres que 0 afin de déterminer la meilleure valeur de λ permettant de normaliser les données par la transformation $Z = (x^\lambda - 1)/\lambda$. Les auteurs présentent des formules d'estimation pour $\lambda = ½, ⅓, ¼$ et $⅙$ de même que la transformation logarithmique $Z = \log x$ qui correspond à $\lambda = 0$. Ces méthodes ont été appliquées aux données obtenues au cours des relevés du hareng en mer du Nord réalisés en 1984 et 1985. L'erreur d'échantillonnage est d'environ 25 % au cours de chacune de ces années. Tant le temps séparant la rencontre de bancs que la taille des bancs peuvent être décrits par des distributions de Weibull à trois paramètres. Les expressions théoriques de la quantité moyenne de poisson, de même que la variance de cette moyenne, sont présentées sous forme de fonctions de paramètres de Weibull. Des simulations informatiques basées sur ces distributions confirment la validité de la procédure d'estimation proposée. Les estimateurs obtenus par le test de Box–Cox sont ceux qui présentent le moins de biais. Le biais s'avère important avec d'autres estimateurs. Ainsi, lorsque les transformations logarithmiques sont appliquées à des données ne présentant pas de distribution normale logarithmique et que le test de Box–Cox indique que $\lambda > 0$, il est alors probable que la quantité moyenne de poissons, et sa variance, sont surestimées.

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Acoustic surveys are conducted to estimate the size of pelagic fish stocks. The echoes from insonified fish are accumulated by an echo-integrator whose output is proportional to the fish density in the acoustic beam. From the fish densities recorded along the survey track, a stock estimate is computed for the whole area. The technique has been well described in published reviews, for example Johannesson and Mitson (1984) and MacLennan and Forbes (1987).

The usual procedure during an acoustic survey is for the survey vessel to steam over a grid of line transects. The variation of echo strength from one transmission to the next is extremely high, so many transmissions are combined to obtain one sample of fish density with moderate variance. Each sample is the accumulated echo-integrator output over a portion of cruise track, usually from 1 to 5 mi long (1 mi = 1.61 km). The width of the insonified water column depends upon the water depth and the transducer beamwidth, perhaps 10–20 m. Thus each sample represents the mean fish density over a thin path, and the paths corresponding to successive samples are continuous.

The statistical analysis of sampling error and variance, in a survey based on line transects, is different from the case of

randomly placed point samples such as might apply, for example, in egg or larval surveys. The survey track is normally structured as a set of regularly spaced transects. Autocorrelation may occur because the samples are from continuous sections of transects. Kimura and Lemberg (1981) have proposed a survey scheme of transects with random starting points. However, this scheme would result in a complicated survey track which is likely to be impracticable (Francis 1985).

In this paper, we consider the sampling error in acoustic stock estimation and how it may be estimated from statistics of the survey data. Estimators are presented for the expected stock size and its variance, for several assumed distributions of the data. The theoretical arguments are illustrated by reference to acoustic surveys for herring in the North Sea.

Review of Previous Work

A relatively simple method of estimating variance is to conduct replicate surveys. The same ship may survey an area at different times or two ships may do this at nearly the same time. The difference between the results of two surveys may be used to estimate the variance, preferably by repeating the replication over a number of subareas to provide sufficient data. However, this variance applies only to the fish population in the areas where the replicate surveys took place. Because of the cost of ship time, it is only practicable to do the replicate experiment in a small part of the total area. The subarea may not be representative of the total area. The analysis is complicated by the possibility of fish moving into or out of the subareas in the time between surveys. It would be much better if the variance could be estimated as a statistic of the survey data, thus avoiding the need for expensive replication.

Aglen (1983) has considered how the error in the acoustic fish abundance estimate depends upon the density of the survey grid. He defined the "degree of coverage" as $D/\sqrt{A}$ where D is the total length of cruise track and A is the area circumscribed by the outermost limits of the transects. The coefficient of variation (CV, the standard deviation divided by the mean) of the stock estimate correlated significantly with the degree of coverage:

$$(1) \quad \mathrm{CV} = 0.5(D/\sqrt{A})^{-0.41}.$$

The individual survey results contributing to equation (1) showed considerable scatter which probably results from differences in the fish distribution during the surveys considered by Aglen. Fish may be scattered as individuals, they may be clumped in shoals, and shoals may be clustered to a greater or lesser extent. For the same degree of coverage, it is reasonable to suppose that the precision will depend upon the homogeneity of the fish distribution.

The significance of the fish distribution has been considered by Gerlotto and Stequert (1983). They developed a computer simulation based on the results of two surveys in which the fish distribution was known to be different, one described as "homogeneous" and the other as "heterogeneous." The CV was more than three times greater for the heterogeneous compared with the homogeneous type of distribution. What is missing, however, is some means of quantifying the homogeneity of the fish distribution, in order to predict the CV for any fish distribution.

Williamson (1982) considered the problem of serial correlation in line transect data. Given a time series of fish density values, $Q_1, Q_2, \ldots, Q_n$, then in the absence of correlation the variance of the sample mean is estimated by the usual formula which assumes that the Q_i are a random sample:

$$(2) \quad \mathrm{Var}\,(\bar{Q}) = \sum_{i=1}^{n} (Q_i - \bar{Q})^2/\{n(n-1)\}.$$

However, when the Q_i are collected consecutively along transects, serial correlation may be significant. Williamson simulated sets of survey data with different levels of correlation. He showed that if the autocorrelation coefficient at lag 1 is less than 0.25, random sampling theory may be used to construct 95% confidence limits on the mean fish density. Var $(\bar{Q})$ is close enough to the true variance for this purpose. When the autocorrelation is significant, a cluster sample estimator due to Kish (1965) may be used instead of Var $(\bar{Q})$. However, the Kish estimator is only valid if the transects are of similar length; the CV of the number of observations per transect must be less than 20%. Francis (1985) pointed out that the cluster sample approach depends also upon stationarity, i.e. the Q_i must all have the same statistical distribution, and in particular, the same population mean. When the distribution is not stationary, the cluster sample method underestimates the true variance.

Acoustic Survey Technique

Data Collection

The echo-integrator data are accumulated over a period of time, known as a "block," normally in the range 10–30 min. One sample is derived from each block, representing the average fish density over the track covered. The research vessel steams over a survey grid which is designed to cover the area of interest, using prior knowledge of fish distribution to make best use of the time available. Samples are collected from successive blocks, except that it may be necessary to interrupt the procedure to trawl, or if the survey is restricted to particular times of day. In the North Sea herring survey, the block length is 30 min. Samples are collected only during daylight, when the fish are in easily detected shoals. Up to 500 samples are collected during the survey, equivalent to a total cruise track of 2500 mi. The area of interest is about 90 000 mi² and extends from the Firth of Forth to the Shetlands (Fig. 1).

Analysis Procedure

One approach is to divide the area of interest into rectangles bounded by lines of latitude and longitude. The samples in each rectangle are assumed to come from a homogeneous distribution. The mean fish density for each rectangle is estimated, and the fish quantity is estimated as the mean density multiplied by the rectangle area. Finally, the total fish quantity for the area of interest is simply the sum of the quantities in the rectangles. In the North Sea herring survey, the rectangles are each 0.5° of longitude by 0.25° of latitude. The number of samples per rectangle is variable, from 1 to 14 in 1985, and averaging about 5. This reflects the irregularity of the cruise track and the need to sample some areas more heavily than others.

In the course of the North Sea surveys, systematic differences have been observed in the size composition of herring taken in trawl catches. This has led to the survey area being divided into five subareas for the purpose of calculating fish target strengths. This does not directly affect the statistical analysis, although the shoal frequency and fish size distributions could change between the subareas. We return to this point later.

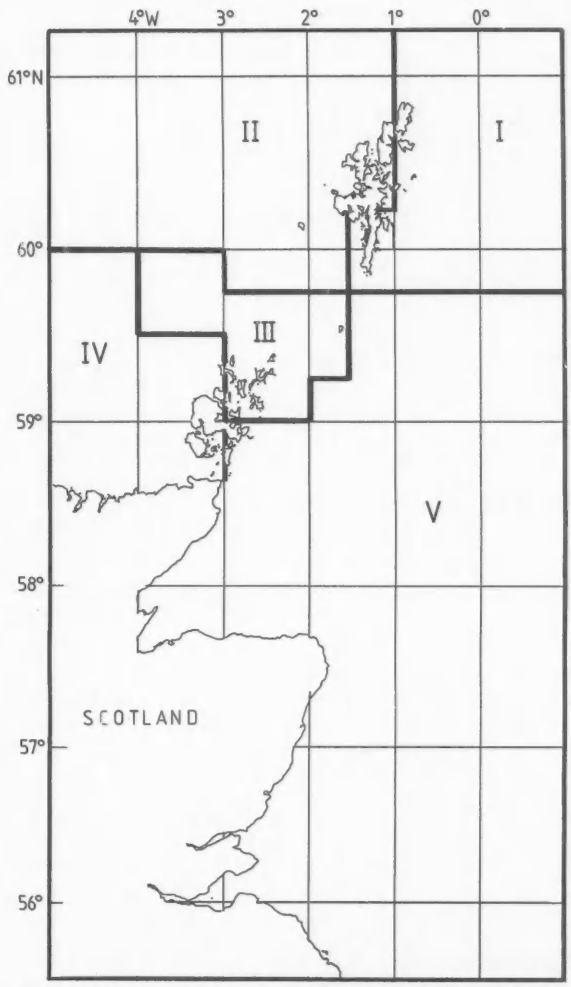

FIG. 1. Area covered during the 1985 North Sea herring survey. The area is divided into five subareas (I–V) on the basis of the size composition of trawl catches.

Partitioning

The survey data consist of observations in two forms. Firstly, there are the original echo-integrator records which include all detected shoals, irrespective of species. Secondly, there are the estimates of the proportion attributed to the target species. The original records are partitioned between the target species and others, using expert knowledge to interpret echo-sounder traces and trawl catches. This procedure has little effect if the target species is a large proportion of the total. Otherwise, it may well result in selected data with different statistical properties. In the 1985 North Sea survey, 78% of the biomass in shoals was identified as herring.

Correlation

The analysis of errors is greatly simplified if the samples are independent of one another, meaning that there is no significant correlation between one sample and the next. This hypothesis may be tested by computing the autocorrelation function, treating the samples recorded during the survey as a time series. Suppose that all shoals encountered are very large, such that at least two samples are recorded while crossing each shoal. These two samples are not independent. Consequently, the autocorrelation function at a lag of one should be significant. To avoid this problem, the length of cruise track corresponding to one sample must be large compared with the horizontal extent of the typical shoal. Moreover, if the spatial distribution of shoals is contagious, the elementary track length must be large compared with the scale of shoal clustering.

There is another cause of significant autocorrelation, connected with the structure of the survey grid. Suppose the ship follows a zigzag pattern from the coast out to sea and back again, which is a common survey design scheme. If the fish are concentrated inshore, the autocorrelation function will show a cyclic pattern with a period corresponding to the zigzag. Again, the correlation will greatly complicate the calculation of variance.

Given the same fish distribution and cruise track, the elementary distance sampling unit (EDSU) will partly determine the amount of correlation. The EDSU is the distance over which the vessel steams while the echo-integrator data are accumulated to give one sample of fish density. In general, the samples should become more independent as the EDSU increases. The ideal EDSU should be long enough to avoid significant autocorrelation, but not so long that fewer independent data are collected, thus losing information about the variability of the data set.

Williamson (1982) has studied echo-integrator data from a survey of Pacific whiting (*Merluccius productus*). He reported significant autocorrelation of the data. However, his EDSU was only about 300 m, corresponding to 1 min of steaming time. The correlograms showed decreasing curves, suggesting that a larger EDSU would lead to more independent data. Smith (1978) has examined the correlation of shoal distribution in the Los Angeles Bight. The shoals were predominantly of northern anchovy (*Engraulis mordax*). His results indicated that independent observations could be obtained with an EDSU of 5 mi or more. Significant autocorrelation was found between samples separated by less than this distance.

In the case of the North Sea herring (*Clupea harengus*), an annual acoustic survey is conducted in the summer. The fish are found in widespread shoals, mostly offshore. The structure of the cruise track is unlikely to contribute significantly to the autocorrelation. The EDSU is about 5 mi. This is very much longer than the typical shoal. Thus we are left with the contagion of the shoal distribution as the most likely source of autocorrelation in this particular case.

The acoustic records from the 1984 and 1985 surveys have been tested for autocorrelation between successive samples, as if the samples were from a time series. The samples in this case are quantities of herring found in 30-min periods. The total data set has been partitioned between species on the basis of the composition of trawl catches and other evidence. However, no herring are observed in a large proportion of the samples, 44% in 1984 and 66% in 1985. Moreover, the zeros tend to be associated; a zero in one 30-min period greatly increases the probability of finding no herring in the next period. The implication is that the herring concentrate in particular areas and they are absent or at too low density to be detected in other areas. This type of nonstationarity may be treated by considering the zero samples and the others separately, as described by Aitchison

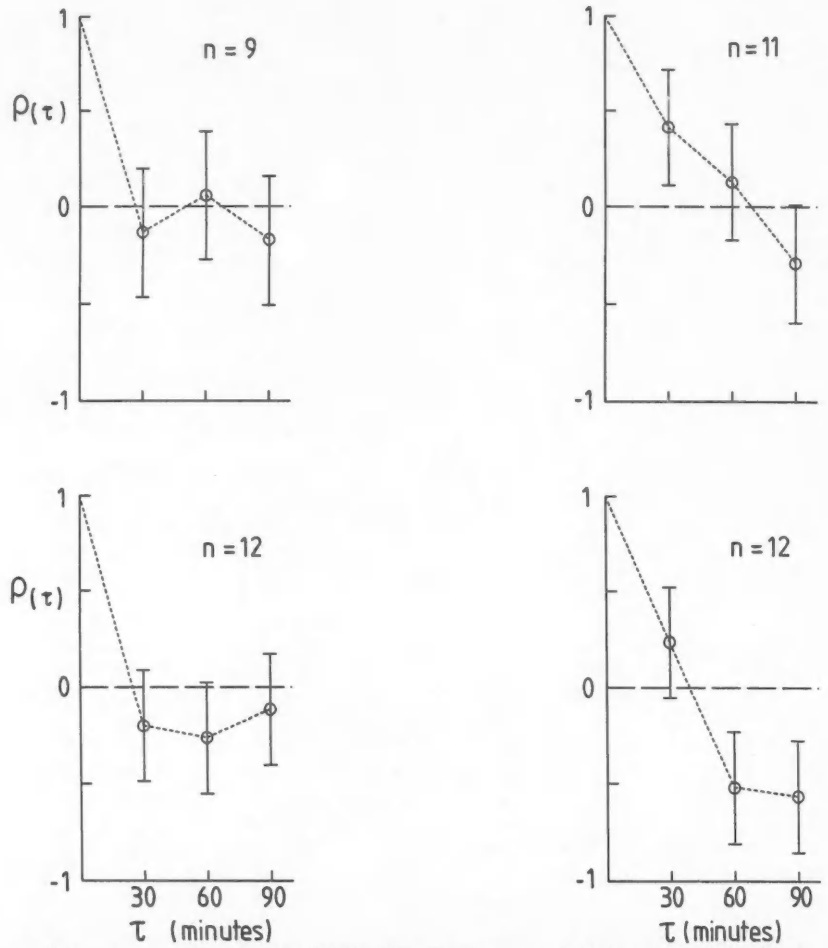

FIG. 2. Correlograms of data from the 1985 North Sea herring survey. $\rho(\tau)$ is the autocorrelation function of herring quantities detected in 30-min blocks; n is the number of consecutive blocks, all containing some herring, in each series. The bars are ± 1 standard deviation ($1/\sqrt{n}$).

(1955). What we now consider, therefore, is whether autocorrelation is significant within the sections of the acoustic record which do not contain zeros.

Figure 2 shows correlograms constructed from the 1985 data. In each case the calculations have been performed for lags up to 33% of the nonzero record length. The standard deviation of each point, under the hypothesis that there is no autocorrelation, is $1/\sqrt{n}$, where n is the number of observations. The correlation coefficients at lags greater than zero are not significant.

A series of data aggregated over 5-min intervals has been extracted from the 1984 records to test for autocorrelation in fish quantities aggregated over short periods. These data have been collected from an area of higher than average density of shoaling fish. Although the data include all shoals, irrespective of species, the proportion of herring is estimated from trawl catch data and other evidence to be 86% by weight. The autocorrelation function has been calculated up to a lag of 12 intervals (60 min) (see Fig. 3). All the points are within 1 standard deviation of zero, and again we conclude that there is no significant autocorrelation.

Another possible source of correlation is the relationship between the shoal size and the intershoal time interval. Correlations between shoal biomass and intershoal intervals have been calculated for five different samples from the 1985 survey; none is significant at the 5% level.

These results support our view that, in the case of the summer herring survey in the North Sea, successive samples accumulated over 30-min intervals are uncorrelated, and the statistical methods of random sample analysis may be applied to the estimation of mean and variance. It is emphasised that this conclusion does not necessarily apply to other acoustic surveys and in particular not to surveys with a repetitive grid pattern which may lead to periodicity in the echo-integrator records.

Estimation Formulae

Power Transformations

Consider the data set Q_i, $i = 1 \rightarrow N$, of fish quantities observed in N blocks of duration T. The Q_i are all greater than

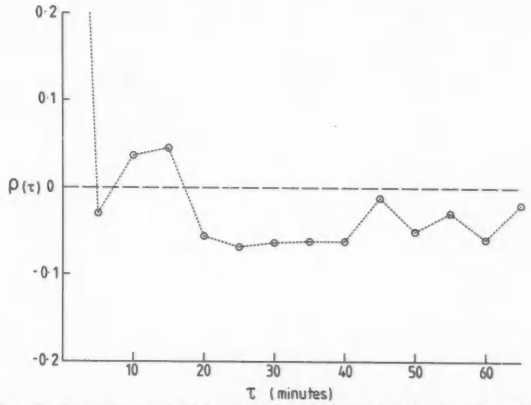

FIG. 3. Correlogram of fish quantities in 5-min blocks from the 1984 North Sea herring survey. The data have been extracted from echo-sounder records during 11 h of survey in an area of relatively high fish density. The standard deviation of the empirical $\rho(\tau)$ is 0.09 (n = 132).

zero and N is 2 or more. We suppose that the Q_i are random samples drawn from a stationary distribution. The following power transformation $Q(\lambda) \rightarrow Z$ is continuous at $\lambda = 0$:

(3a) $Z_i = (Q_i{}^{\lambda} - 1)/\lambda$ for $\lambda > 0$

(3b) $= \log Q_i$ for $\lambda = 0$.

We suppose that for some given λ, the Z_i are normally distributed. An alternative transformation, not continuous at $\lambda = 0$, is

(3c) $Y_i = Q_i{}^{\lambda}$ for $\lambda > 0$

(3d) $= \log Q_i$ for $\lambda = 0$.

The Z-transforms (3a,3b) are useful for the Box–Cox analysis of normality to which we return later. Estimation formulae for discrete λ values are more usually expressed in terms of the Y-transforms (3c,3d). Let m and v be respectively the sample mean and residual sum of squares of the Y_i:

(3e) $m = \Sigma\, Y_i/N$

(3f) $v = \Sigma\, (Y_i - m)^2$.

The formulae described below may be used to estimate $\hat{Q}$, the true mean of the sample, and the variance of this mean, $\hat{V}$. The selected estimators are accurate if the transformed data are normally distributed; otherwise, they will be subject to biasses of uncertain magnitude.

Estimators for the square-root, cube-root, and log normal cases have been derived by Hoyle (1968). There is an error in Hoyle's expression for the variance of the cube-root normal which has been corrected in our equation (5b).

Square-root normal (λ = 1/2)

(4a) $\hat{Q} = m^2 + v/N$

(4b) $$\hat{V} = \frac{4vm^2}{N(N-1)} + \frac{v^2}{N^2} - \frac{v^2}{N^2-1}\left(1 - \frac{2}{N} + \frac{3}{N^2}\right)$$

Cube-root normal (λ = 1/3)

(5a) $\hat{Q} = m^3 + 3mv/N$

(5b) $$\hat{V} = \frac{9m^4 v}{N(N-1)} + \frac{9m^2 v^2}{(N-1)^2}\left[\left(1 - \frac{1}{N}\right)^2 - \frac{N-1}{N+1}\left(1 - \frac{4}{N} + \frac{5}{N^2}\right)\right] + \frac{3v^3}{N(N^2-1)\,(N+3)}\left(3 - \frac{6}{N} + \frac{5}{N^2}\right)$$

Fourth-root normal (λ = 1/4)

(6a) $\hat{Q} = m^4 + 6m^2\, v/N + 3\,(N-1)\,(v/N)^2/(N+1)$

(6b) $$\hat{V} = \hat{Q}^2 - m^8 - \frac{m^6\, v}{N-1}\left(12 - \frac{28}{N}\right) - \frac{m^4\, v^2}{N^2-1}\left(\frac{210}{N^2} - \frac{180}{N} + 42\right) - \frac{m^2\, v^3}{(N^2-1)\,(N+3)}\left(36 - \frac{252}{N} + \frac{540}{N^2} - \frac{420}{N^3}\right) - \frac{v^4}{(N^2-1)\,(N+3)\,(N+5)}\left(9 - \frac{36}{N} + \frac{126}{N^2} - \frac{180}{N^3} + \frac{105}{N^4}\right)$$

Sixth-root normal (λ = 1/6)

(7a) $$\hat{Q} = m^6 + \frac{15\, m^4\, v}{N} + 45\, m^2\, \frac{N-1}{N+1}\left(\frac{v}{N}\right)^2 + \frac{15\,(N-1)^2}{(N+1)\,(N+3)}\left(\frac{v}{N}\right)^3$$

(7b) $$\hat{V} = \hat{Q}^2 - m^{12} - \frac{6\, m^{10}\, v}{N-1}\left(5 - \frac{11}{N}\right) - \frac{45\, m^8\, v^2}{N^2-1}\left(7 - \frac{30}{N} + \frac{33}{N^2}\right) - \frac{60\, m^6\, v^3}{(N^2-1)\,(N+3)}\left(23 - \frac{147}{N} + \frac{315}{N^2} - \frac{231}{N^3}\right) - \frac{225\, m^4\, v^4}{(N^2-1)\,(N+3)\,(N+5)}\left(11 - \frac{92}{N} + \frac{294}{N^2} - \frac{420}{N^3} + \frac{231}{N^4}\right) - \frac{270\, m^2\, v^5}{(N^2-1)\,(N+3)\,(N+5)\,(N+7)}$$

$$\left(5-\frac{55}{N}+\frac{230}{N^2}-\frac{490}{N^3}+\frac{525}{N^4}-\frac{231}{N^5}\right)$$

$$-\frac{45\,v^6}{(N^2-1)\,(N+3)(N+5)(N+7)(N+9)}$$

$$\left(5-\frac{30}{N}+\frac{165}{N^2}-\frac{460}{N^3}+\frac{735}{N^4}-\frac{450}{N^5}+\frac{231}{N^6}\right)$$

Log normal ($\lambda = 0$)

(8a) $\hat{Q} = \exp(m)\, G_N\,[0.5\, v/(N-1)]$

(8b) $\hat{V} = \hat{Q}^2 - \exp(2m)\, G_N\,[v\,(N-2)/(N-1)^2]$.

The function $G_N(u)$ is computed from the following algorithm:

(8c) $G_N(u) = 1 + (N-1)\,u/N$

(8d) $g = (N-1)^3\,u^2/[2N^2\,(N+1)]; j = 3$

(8e) $G_N(u) \longrightarrow G_N(u) + g$

(8f) $g \longrightarrow g\,(N-1)^2\,u/[Nj\,(N+2j-3)]; j \longrightarrow j+1$.

The equations (8e,8f) are repeated until convergence, when g is very close to zero.

The Box–Cox Test

The estimation formulae (4–8) will only be unbiased if the transformed variable has a normal distribution. The formulae then represent minimum variance unbiased estimators (MVUEs). When an inappropriate transformation has been used, the values of the estimators are uncertain and bear little relationship to the true values. It is therefore necessary to determine which value of λ is the most appropriate choice for a given data set. This may be done using a test for normality of the transformed variable devised by Box and Cox (1964). The Box–Cox function is

(9) $$L(\lambda) = -(N/2)\log\left\{\sum_{i=1}^{N}(Z_i-\bar{Z})^2/N\right\} + (\lambda-1)\sum_{i=1}^{N}\log(Q_i).$$

The maximum of the $L(\lambda)$ curve, at λ_m, indicates the value of λ for which the transformed data are most nearly matched to the normal distribution. Since $L(\lambda)$ is distributed as chi squared, 95% confidence limits on λ_m are given by the solution of

(10) $L(\lambda) = L(\lambda_m) - 1.92$.

Nonstationary Distributions

If the species of interest is absent from part of the survey area, correlated sequences of zero blocks are liable to occur and the data distribution is not stationary. However, if it is assumed that conditions are stationary within the regions where the fish are found, the estimation formulae (4–8) may still be applied, by treating the zero and nonzero data separately. Consider the distribution

(11) $F_1(Q) = p$ for $Q = 0$
$= (1-p)\,F(Q)$ for $Q>0$

where $F(Q)$ is such that under the transformation $Q(\lambda) \longrightarrow Z$, the Z are approximately normally distributed. Let

$\hat{Q} = E(Q \mid Q>0)$
$\hat{V} = \mathrm{Var}(\hat{Q} \mid Q>0)$.

For a sample of size M, containing N values greater than zero, Pennington (1983) gave the following formulae for $\hat{Q}_r$, the estimate of the mean, and $\hat{V}_r$, the estimated variance of the mean:

(12a) $\hat{Q}_r = N\hat{Q}/M$

(12b) $\hat{V}_r = N(M-N)\hat{Q}^2/[M^2(M-1)] + N(N-1)\,\hat{V}/[M(M-1)]$.

The estimate of p is N/M. Thus the appropriate formulae from equations (4–8) may be used to obtain $\hat{Q}$ and $\hat{V}$ from calculations with the nonzero data. Then equations (12) may be used to obtain the overall estimate of fish quantity, taking account of those areas where no fish are found.

Modelling the Occurrence of Shoals

A model of shoal encounters has been developed to investigate the statistics of surveys which depend upon accumulating measurements from individual shoals. Inspection of the data extracted from echo-sounder records, as described above, suggests that the three-parameter Weibull distribution would be an appropriate statistical model to describe both the time between shoals, T, and the size of individual shoals, S. Strictly, S is the size of the observed part of the shoal; the vertical echo-sounder will detect only a section through a large shoal. The Weibull distribution is

(13) $P[X<x] = 1-\exp\{-\log_e(2)\,((x-c)/m)^b\}\; c<x<\infty$

where b is the shape parameter, m is the scale parameter, and c is a threshold parameter which, in the case of time distributions, has been fixed at the practical limit of observation on the paper record, 0.25 min. In the case of size distributions, the choice of c depends upon the smallest likely size of shoal, or the smallest echo-trace which could be identified and measured as a shoal of interest. Throughout this paper, the subscripts 1 and 2 are used with b, m, and c to denote the time and size parameters, respectively. Thus, six parameters are required to define the shoal observations, three for the encounter time and three for the shoal size.

Having determined appropriate distributions for T and S, the model may be used to generate simulated survey data for statistical investigation. A series of pairs of random values is generated, each pair giving the time to the next shoal and its size. The simulated fish quantities are combined into 30-min blocks. The blocks are then combined into rectangles, and formulae for estimating the mean and variance of the fish stock prediction may then be tested against the statistics of results from repeated simulations.

The 1984 Survey

The value of c_2 was chosen at the lowest practicable limit of observation, the smallest echo-sounder mark which could be identified as a shoal. Data were sampled from two continuous periods of echo-sounder recording. The size of each shoal was estimated as the product of the horizontal and vertical extent of the shoal as measured on the echo-sounder paper record. This relatively crude procedure estimates the volume of the shoal but does not take account of the fish density within. However, the Weibull parameters for a matching distribution were estimated

TABLE 1. Weibull parameters for distributions of shoal encounter times and shoal sizes. c_1 and c_2 are determined by the limits of observation. The other parameters are obtained by fitting the distributions to data from the North Sea herring surveys. D_m indicates the goodness-of-fit. λ_m is the best estimate of the index for power law transformation, obtained from Box-Cox calculations on simulated survey data.

	Distribution		
Data set	A 1984, all shoals	B 1985, all shoals	C 1985, large shoals
m_1	3.5	7.0	13.0
b_1	0.6	0.6	0.6
c_1	0.25	0.25	0.25
m_2	28	1.0	2.0
b_2	0.6	0.5	0.5
c_2	4.0	1.5	2.5
D_m	<1	<1	0.95
λ_m	0.22	−0.08	−0.18

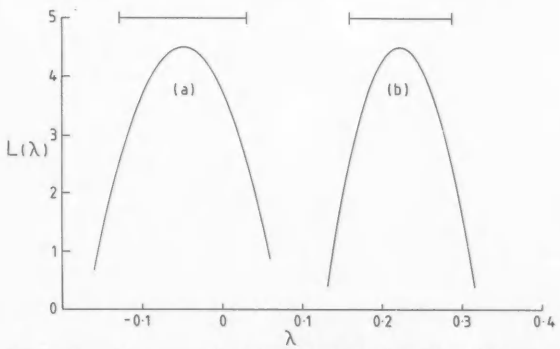

FIG. 4. Box–Cox curves for (a) actual blocks from the 1984 survey and (b) simulated block data. Weibull distribution A (ref. Table 1) was used in the simulation. The horizontal bars indicate 95% confidence limits on the positions of the curve maxima.

using maximum likelihood estimation. The results are shown in column A of Table I. The units of shoal size are arbitrary. The goodness-of-fit was examined using the Kolmogorov–Smirnoff (KS) test. D_m is the maximum difference between the fitted and empirical cumulative distributions, multiplied by the square root of the number of shoals in the sample. The fitted distributions are well matched to the observations ($D_m < 1$; $P = 0.9$).

We generated 500 blocks of simulated survey data using the fitted Weibull distributions and performed Box–Cox calculations to compare the simulation with the actual data (253 non-zero blocks) from the 1984 survey. The results are shown in Fig. 4. The maxima occur at different positions, $\lambda_m = 0.22$ and -0.05 for the simulated and actual data, respectively. The 95% confidence limits on λ_m do not overlap, indicating a significant difference in skewness between the two data sets.

Figure 5a shows the quantities of herring found in 30-min blocks during the 1984 survey. The data have been log-transformed for ease of presentation. While there is some clustering at small block values, the histogram shows no obvious skewness. Figure 5b has been derived from simulated data, using the Weibull distributions in column A of Table 1, and thus includes the contribution from all shoaling fish. In this case, the distribution is strongly skewed to the left.

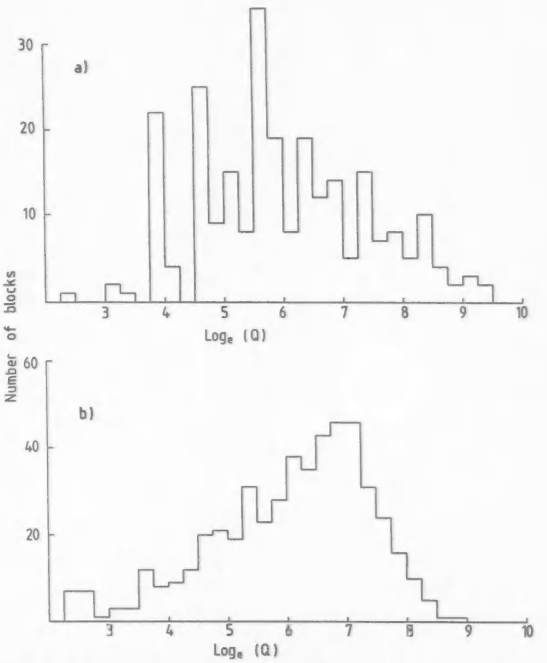

FIG. 5. Histograms of log-transformed data from (a) the 1984 survey and (b) simulation using Weibull distribution A. Q is the fish quantity per 30-min block.

One limitation of our model is that all shoals larger than the threshold c_2 are included when fitting the Weibull distributions. There is no provision for partitioning the data between the target species and other shoaling fish. In the case of the actual survey, this is done on the basis of trawl catches and other indirect evidence. At present, the identification of echo-traces, on the basis of acoustic information alone, is often uncertain and subjective. Our model will be most easily applied when the identity of shoals is known, or when the aim is to determine the quantity of shoaling fish, irrespective of species.

On the other hand, the discrepancy between the model and the actual survey may have arisen from the crude method of estimating shoal size from the echo-graph. This method depends upon the fish density within shoals being independent of the shoal size. The distribution of the actual survey data is not significantly different from the log-normal, since the confidence limits on λ_m include zero.

The 1985 Survey

The measurements of shoal size were now taken from the echo-integrator record to overcome the limited accuracy of direct measurements of the echo-sounder marks.

Examination of the 1984 results suggests that many of the smallest shoals, those just above the c_2 threshold, are not herring. An increase in the threshold should exclude many tiny aggregations which are not characteristic of herring, although the larger non-herring shoals would still be included in the analysis. For the 1985 data, the smallest likely size of a herring shoal is considered to be 2.5, about 70% above the minimum detectable shoal size of 1.5 units. Note that the different size units used in the 1984 and 1985 analyses mean that the respec-

TABLE 2. Box–Cox λ_m values for data from the 1984 and 1985 North Sea herring surveys, and 95% confidence limits.

Year	Area	λ_m
1985	I	$+0.06 \pm 0.26$
	II	-0.19 ± 0.24
	III	$+0.16 \pm 0.72$
	IV	$+0.03 \pm 0.23$
	V	-0.17 ± 0.17
	All	-0.11 ± 0.10
1984	All	-0.05 ± 0.08

TABLE 3. Comparison of optimum λ values for the 1985 data and two simulated data sets which have different shoal size thresholds c_2. Other Weibull parameters for the simulation are from distribution B in Table 1. The limits on λ_m show the 95% confidence interval.

Data set	λ_m
Actual 1985	-0.11 ± 0.10
Simulated $c_2 = 1.5$	-0.08 ± 0.08
Simulated $c_2 = 2.5$	-0.18 ± 0.09

tive c_2 values are not directly comparable between years. Weibull distributions have been fitted to the empirical 1985 shoal data for both $c_2 = 1.5$ and 2.5, and the results are given in columns B and C of Table 1.

The area of the 1985 survey was divided into five subareas on the basis of observed differences in the size composition of trawl catches. This was done primarily for the calculation of the fish target strength which depends upon the fish size. To determine whether there were also significant differences in shoal distribution, we performed Box–Cox calculations on the herring quantities observed by block in the five subareas. The results are shown in Table 2. The λ_m are not significantly different. Therefore, for the purposes of this paper, there is nothing to be gained from separate treatment of the subareas. We shall confine attention to the distributions in Table 1 which have been fitted to pooled data from the whole survey area.

Box–Cox calculations have also been performed on simulated block data generated from the fitted Weibull distributions. These are compared with the actual 1985 survey data (Table 3). The simulated data for $c_2 = 1.5$ and 2.5 bracket the actual survey data; the three λ_m are not significantly different. We conclude that the method used in 1984 to extract shoal size information is unsatisfactory; the shoal size should always be based on the echo-integrator record, if it is available.

Mean and Variance Estimation

To test alternative estimators of fish quantity, the shoal encounter model was used to generate a series of 30-min blocks, as described above. A given number, M, of these blocks were grouped together to represent the data in one rectangle. The mean and the variance of the mean were then calculated for each rectangle. The calculations were performed firstly on the untransformed data, including zero points, and then on the transformed nonzero data, using the method of Pennington (1983) to treat the zero values as described earlier.

The mean and variance estimates were then aggregated over several rectangles to simulate about 100 blocks of data per survey. This procedure was repeated many times so that the statistical properties of the aggregates of mean fish quantity estimates could be examined.

The statistics of the aggregate fish quantity observed in a block may be deduced from the properties of the Weibull distribution. Let $\bar{q}$, $\bar{\tau}$, and $\bar{n}$ be the respective true means of fish quantity per shoal, time between shoals, and the number of shoals encountered in time T; let $\sigma_q^2, \sigma_\tau^2$, and σ_n^2 be the corresponding variances.

From a well-known result of renewal theory, as the observation time tends to infinity:

$$\bar{n} \longrightarrow T/\bar{\tau} \tag{14a}$$

$$\sigma_n^2 \longrightarrow (\sigma_\tau^2/\bar{\tau}^2)\,(T/\bar{\tau}). \tag{14b}$$

If $Q(T)$ is the total fish quantity encountered in a block of duration T, then

$$Q(T) = \sum_{i=1}^{n} q_i \tag{15}$$

where q_i is the quantity of fish in the ith shoal encountered. The q_i are independent and have identical distributions, so the true mean is

$$Q_t = E[Q(T)] = E(n)\,E(q) = T\,\bar{q}/\bar{\tau} \tag{16}$$

and the corresponding variance is

$$\begin{aligned} V_t &= \mathrm{Var}\,[Q(T)] = \sigma_q^2\,\bar{n} + \sigma_n^2\,\bar{q}^2 \\ &= (T/\bar{\tau})\,(\sigma_q^2 + \sigma_\tau^2\,\bar{q}^2/\bar{\tau}^2). \end{aligned} \tag{17}$$

These equations are approximations; they are assymptotically true as the length of the survey track increases to infinity.

From the properties of the Weibull distribution:

$$\bar{\tau} = (m_1/b_1\,\Gamma(1/b_1)/[\ln(2)]^{1/b_1} + c_1 \tag{18}$$

$$\sigma_\tau^2 = m_1^2\,[\Gamma(1+2/b_1) - \Gamma^2(1+1/b_1)]/[\ln(2)]^{2/b_1}. \tag{19}$$

Similar expressions give $\bar{q}$ and σ_q^2 as functions of m_2, b_2, and c_2. Substituting in (16) and (17) gives formulae for the true mean and variance of the fish quantity per block in terms of the six Weibull parameters and the block duration T.

If the survey covers R rectangles and N blocks per rectangle, the total fish quantity is proportional to

$$\tilde{Q} = \sum_{i=1}^{R} Q_{ti} \tag{20}$$

and the variance of $\tilde{Q}$ is

$$\tilde{V} = RV_t/N. \tag{21}$$

Results

Figures 6 and 7 show a comparison of the theoretical mean and variance, given by equations (20–21), with the sample mean and variance obtained from several hundred simulation runs and for various sets of Weibull parameters near to the values fitted to the 1984 and 1985 survey data. The agreement between the predicted and sample values is extremely good. This justifies the use of equations (20–21) as theoretical benchmarks against which to compare other estimators.

Figure 8 illustrates the properties of various transformations applied to data generated from shoal distribution A. The graphs on the left of the figure show the ratio of the estimated and theoretical fish quantities plotted against N, the number of blocks per rectangle.

There is some bias in $\hat{Q}$ at $\lambda = 1/6$ and a very significant amount at $\lambda = 0$. The bias increases with N. Note that the scale of the $\lambda = 0$ graphs is smaller than the others.

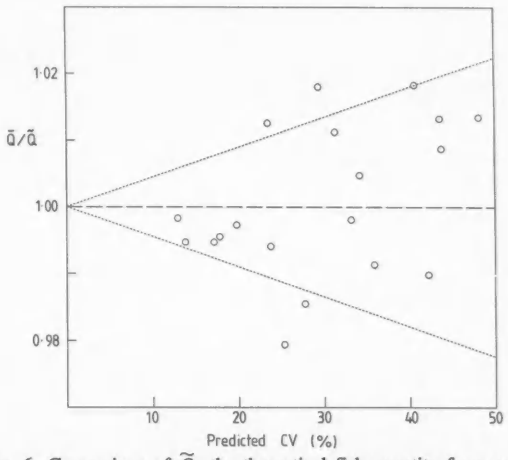

FIG. 6. Comparison of $\widetilde{Q}$, the theoretical fish quantity from equation (20), with $\hat{Q}$, the sample mean of 500 simulated surveys. The ratio $\tilde{Q}/\widetilde{Q}$ is plotted against the theoretical CV from (20–21). Each point represents 25 000 h of simulated observation using one set of Weibull parameters. The dotted lines show the range ±1 standard error of $\tilde{Q}/\widetilde{Q}$.

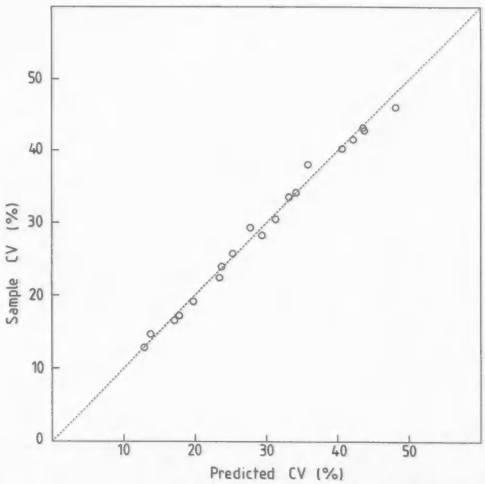

FIG. 7. Comparison of the CV of fish quantity from equations (20–21) with that from the sample variance of 500 simulated surveys as in Fig. 6. The dotted line represents one-to-one correspondence.

The graphs on the right-hand side of Fig. 8 compare the estimated and theoretical variances. Again, there is a large bias at λ eq 0, especially for $N>3$. On the other hand, the $\lambda = 1/4$ plots have the smallest overall error in the variance estimate and negligible bias in $\hat{Q}$. λ_m is 0.22 for the data simulated using distribution A, which is consistent with the results in Fig. 8. This suggests that if the Box–Cox calculation shows that λ_m is significantly greater than zero, the use of log transformations may result in a significant overestimate of the fish quantity and a large bias, probably an overestimate, in the estimate of variance.

Figure 9 shows a similar set of plots for distribution B. In this case, λ_m is -0.05, not significantly different from zero. There is no longer a significant bias in $\hat{Q}$ at small λ, nor indeed at any λ. The variance estimate for $\lambda = 0$ now has the smallest overall error. Again, the analysis of transformations is consistent with the Box–Cox calculation, indicating similar values of λ close to zero as the best choice for the estimation of mean and variance. The block data simulated using distribution B have a distribution not significantly different from the log-normal.

A similar exercise has been performed on distribution C. The results are not significantly different from those in Fig. 9.

The methods described here have been applied to data from the 1984 and 1985 surveys. The log-transform is the most appropriate in both cases. The results are shown in Table 4. The precision is close to 25% in both years, despite the different proportions of zero blocks. It is emphasised that this uncertainty arises from the statistical sampling error alone. It does not include other sources of error in the fish stock estimate, such as uncertainty in the fish target strength and in the partitioning between species.

Bias in the Variance Estimate

If the true population variance is known, then the true variance of the sample mean is

$$\text{Var}(\hat{Q}) = [\text{True population variance}]/N.$$

However, when using MVUEs, we find that

$$\text{Var}(\hat{Q}) < [\text{Estimated population variance}]/N$$

where N is the sample size and the "estimated population variance" is found by the method of Hoyle (1968). If N is large, it can be shown that the difference between the two equations, ΔV, decreases as N increases. When N is small, the behaviour of ΔV is less predictable. For the general transformation, the calculation of ΔV is difficult. As an example, consider the relatively simple case of the cube-root transform for which

$$\Delta V = 6V^3 (N-2)/[N^3(N+1)(N+3)].$$

For $N = 2$, this is zero, but for $N = 3$, $\Delta V = V^3/108$. For $N = 5$, the average number of blocks per rectangle in the 1985 survey, the difference ΔV is $0.003\ V^3$. This is not a serious discrepancy.

In Fig. 8 and 9, $\lambda = 1/4$ and 0 are respectively the values closest to λ_m, and so the corresponding graphs represent the MVUE variance. These graphs exhibit the expected behaviour, namely that the absolute difference between $\hat{V}$ and $\widetilde{V}$ is greatest at $N = 2$ or 3 and then decreases as N increases.

Conclusions

Acoustic surveys of North Sea herring have shown that the fish are concentrated in particular areas and absent from others. Thus the fish distribution is not stationary over the whole survey area. However, if the distribution is stationary in the areas where fish are found, the total fish quantity and its variance may be estimated by considering separately those areas with fish and those without.

The significance of autocorrelation in line transect data depends on the time or distance over which the data are accumulated into elementary sampling units (blocks). In the case of the North Sea surveys, the 30-min block interval, corresponding to 5 mi of cruise track, is sufficient to avoid significant autocorrelation between successive samples in the areas where fish are found. The methods of random sample analysis may

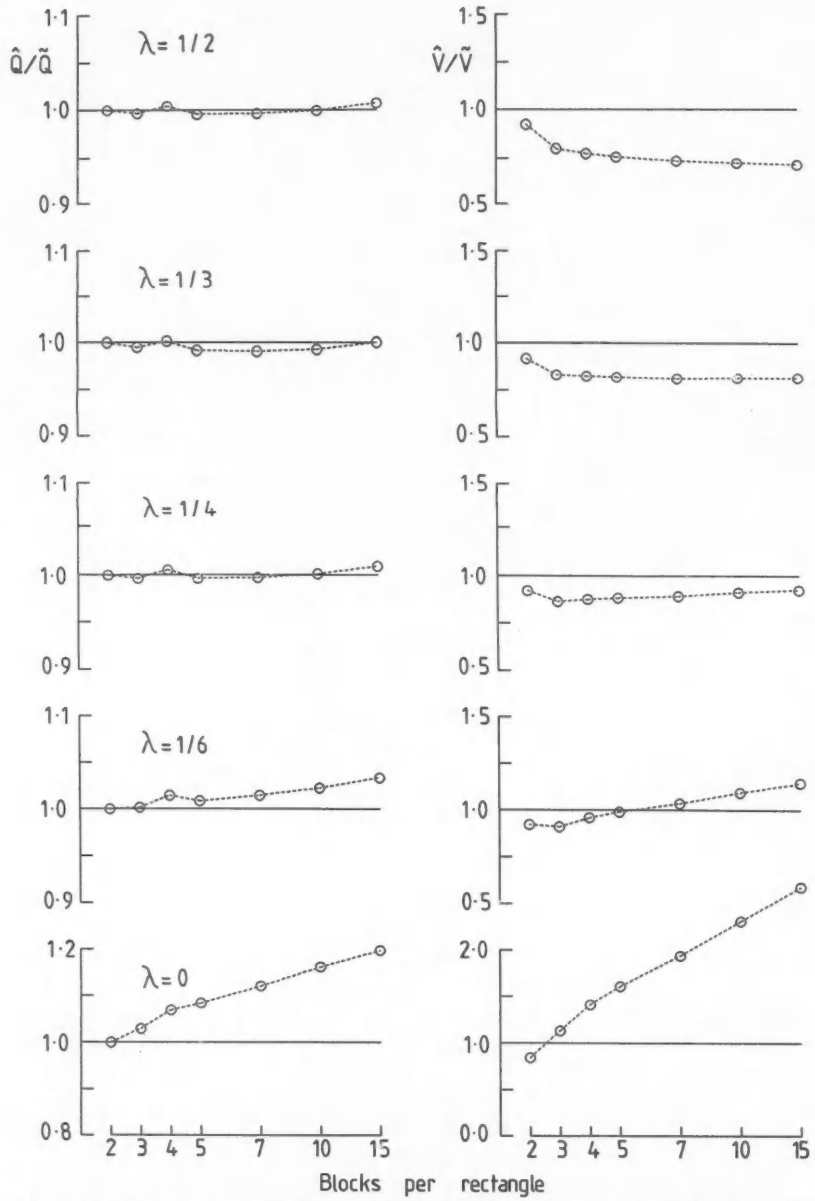

FIG. 8. Comparison of estimated and theoretical statistics of the fish quantity. $\hat{Q}$ and $\hat{V}$ are the estimates from equations (4–8). They are averages over 500 simulated surveys using Weibull distribution A (ref. Table 1). λ is the power transform parameter. The theoretical $\tilde{Q}$ and $\tilde{V}$ are from equations (20–21).

then be used to obtain efficient and unbiased estimators of mean and variance.

The distribution of the nonzero data is highly skew, as is often the case with data from fish surveys. A power law transformation may be applied to normalize the data. Ideally, the power law index λ should be equal to the Box–Cox parameter λ_m which is calculated from the nonzero blocks. However, the estimation of mean and variance from the empirical data is not trivial in the general case. We have presented equations for the special cases $\lambda = 1/2, 1/3, 1/4$, and $1/6$ and for $\lambda = 0$ which is the log transform. The transformation should be performed using the special case closest to λ_m. This theory assumes stationarity in those areas where fish are found.

We have developed a statistical model of shoal encounters to test the proposed estimators of mean and variance. The Weibull distribution may be used to describe both the time between encounters and the observed shoal size. The Weibull distributions are fitted to empirical distributions of shoal occurrences extracted from the acoustic records. The model has been used to simulate survey data. Theoretical expressions are presented

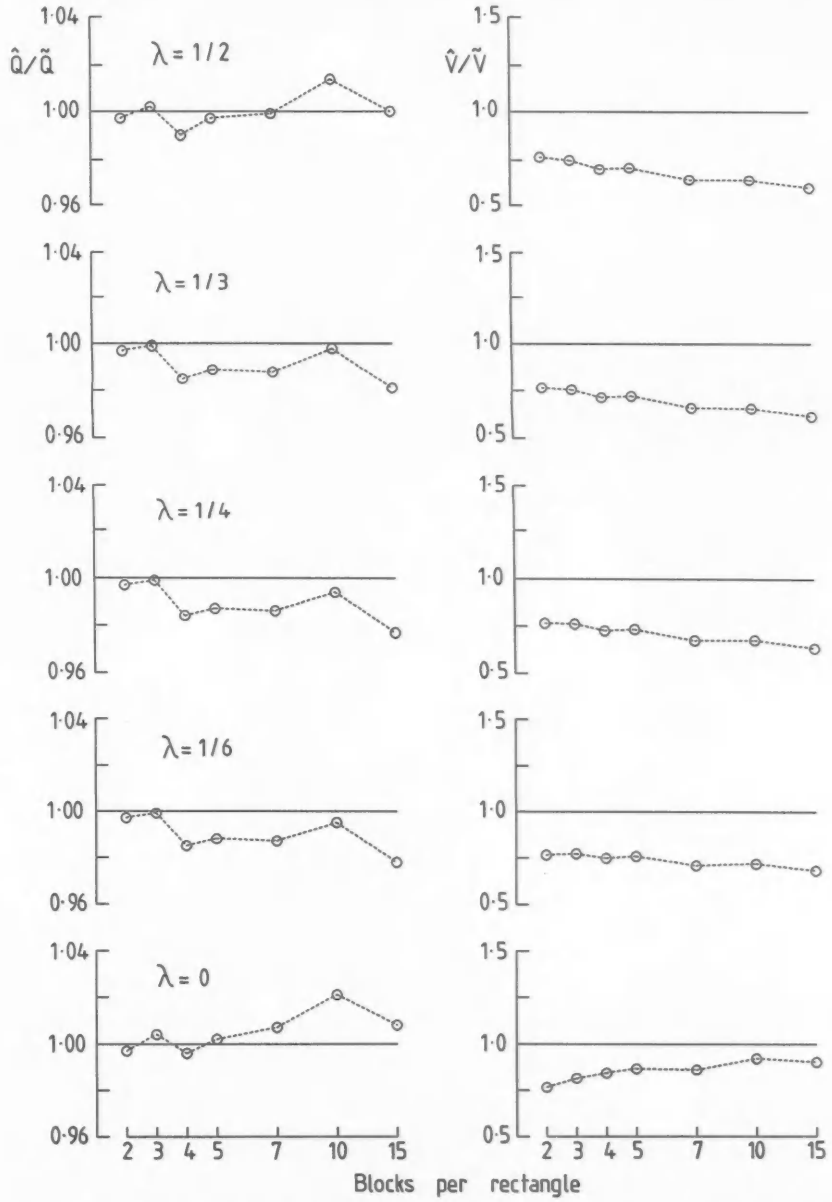

FIG. 9. Comparison of estimated and theoretical statistics of the fish quantity, as in Fig. 8 but using Weibull distribution B.

which give the mean and variance of fish quantity as functions of the Weibull parameters. These expressions have been compared with the sample mean and variance obtained by repeated operation of the model, and the agreement is very good over a wide range of Weibull parameters.

Our model does not take account of the partitioning of acoustic data between species, except where this is done on the basis of identifying individual shoals.

The initial work on the model used crude estimates of shoal size obtained from echo-sounder marks on the paper record. This was unsatisfactory; the model predicted a block data distribution very different from the empirical one. The later work used shoal measurements from the echo-integrator record, leading to model and empirical block distributions which were not significantly different. The effect of changing the threshold shoal size was examined, since it was believed that the very

TABLE 4. Analysis of North Sea herring survey data. The confidence interval (at 95% level) is expressed as a percentage of the stock prediction.

Year	Nonzero blocks	Zero blocks	Confidence interval (%)
1984	253	199	24.8
1985	170	328	25.3

small marks were not herring. However, the simulated data distributions generated from thresholds of 1.5 and 2.5 were not significantly different from one another or from the empirical distribution. There do not seem to be large differences between the unpartitioned data, which include all shoaling fish, and the partitioned data which are supposed to be herring only.

The simulations confirmed that if λ is close to λ_m, then the equations give an unbiased estimate of the fish quantity, together with an estimate of variance which is more efficient than the sample variance and sufficiently accurate for most purposes. However, if the wrong value of λ is used in the transformation, large biases may occur in both the mean and variance estimates. This problem may arise particularly if it is assumed that the data are log-normally distributed, implying the transformation $\lambda = 0$. If λ_m is in fact significantly greater than zero, the log-normal estimators will be biased; they will overestimate the variance and, to a lesser extent, the mean.

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Production by the Amphipod *Pontoporeia hoyi* in South Bay, Lake Huron

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Johnson, M. G. 1988. Production by the amphipod *Pontoporeia hoyi* in South Bay, Lake Huron. Can. J. Fish. Aquat. Sci. 45: 617–624.

Pontoporeia hoyi was the dominant profundal benthic macroinvertebrate comprising 70–90% of animals collected and averaging close to 2100·m^{-2} in the main profundal basin of South Bay, Lake Huron. Almost all production occurred in the profundal zone (1.15 g·m^{-2}·yr^{-1}) compared with the sublittoral zone (0.03 g·m^{-2}·yr^{-1}), attributable to a preference by older *P. hoyi* for cooler temperature. Production/biomass (*P/B*) averaged 1.4·yr^{-1} in the profundal zone at mean annual temperature of 4.7°C and 3.1·yr^{-1} at 7.5°C in the sublittoral zone. Instantaneous growth rates were the same as in the Bay of Quinte and adjacent Lake Ontario where production was six times that in South Bay. A similar difference in phytoplankton production occurred between the areas. Differences in biomass accounted for variation in *P. hoyi* production in the two areas. The South Bay data provide further evidence that benthic macroinvertebrate biomass reflects production consistent with values of *P/B* determined by temperature.

Pontoporeia hoyi était l'espèce de macroinvertébrés benthiques de la zone profonde la plus abondante des espèces animales prélevées dans le bassin de la baie South (lac Huron). Ces animaux représentaient de 70 à 90 % des prélèvements et leur densité moyenne atteignait presque 2 100 individus·m^{-2}. Presque toute la production était notée dans la zone profonde (1,15 g·m^{-2}· a^{-1}) comparativement à la zone du sous-littoral (0,03 g·m^{-2}· a^{-1}). Ce phénomène a été attribué à la préférence des *P. hoyi* plus âgés pour les températures plus froides. Le rapport production/biomasse (*P/B*) s'élevait en moyenne à 1,4·a^{-1} dans la zone profonde, où la température annuelle moyenne était de 4,7°C, et à 3,1·a^{-1} dans la zone sous-littorale, où la température était de 7,5°C. Les taux de croissance instantanés étaient les mêmes que ceux notés dans la baie de Quinte et dans la partie adjacente du lac Ontario où la production était six fois supérieure à celle de la baie South. Un écart semblable de la production du phytoplancton a été noté entre ces deux zones. Des différences de biomasse expliquaient la variation de la production de *P. hoyi* à ces deux endroits. Les données obtenues à la baie South renforcent l'hypothèse que la biomasse des macroinvertébrés benthiques reflète un niveau de production correspondant à des valeurs de *P/B* elles-mêmes déterminées par la température.

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The profundal zone comprises most of the area of the St. Lawrence Great Lakes. Profundal benthos is dominated by the amphipod *Pontoporeia hoyi*, with summer averages of 60–80% of total macroinvertebrates in the upper lakes and 50% in Lake Ontario (Cook and Johnson 1974). While there is considerable data on distribution and abundance of *P. hoyi* in the Great Lakes, production of this important species has been measured rarely (Johnson and Brinkhurst 1971; Lubner 1979; Winnell and White 1984), and measurement of biomass is often omitted in benthic macroinvertebrate surveys. Matusek (1978) and Hanson and Leggett (1982) have shown the value of benthic macroinvertebrate biomass as a preditor of fish yield. Further understanding of the relationship between biomass and production is needed to improve these models, for example, the correlation of turnover rate (production/biomass or *P/B*) with mean annual bottom temperature at stations in the Bay of Quinte and Lake Ontario (Johnson and Brinkhurst 1971).

A further opportunity to study production dynamics in *P. hoyi* was provided from an intensive survey of benthic macroinvertebrate communities of South Bay, Lake Huron, by staff of the South Baymouth Fisheries Research Station (Ontario Ministry of Natural Resources) in 1980–82. South Bay is 86 km^2 in area, has a maximum depth of 59 m, and is separated from Lake Huron by a shallow, narrow channel. The profundal benthic macroinvertebrate community is typical of those observed in Lake Huron and Georgian Bay in which *P. hoyi* comprises 70–90% of macroinvertebrate numbers (Thomas et al. 1973; Loveridge and Cook 1976). *Pontoporeia hoyi* has a 2-yr life cycle with strong cohorts produced each year in the inner, main basin (Cooper 1964: J. Reckahn, unpubl. data). These new collections, made from early May through October, were expected to provide adequate time series for estimation of production from suitable growth models and biomass measurements. The practical application of results is in examining rela-

tionships among biomass, growth, production, and environmental factors such as temperature and food supply which have potential value in empirical models of fish production.

Methods

Duplicate samples were collected at approximately monthly intervals from May to October in 1980 and 1981 at 30 stations (Fig. 1) with depths of 5–48 m. Five stations represented the profundal zone (>25 m), 10 were from the sublittoral zone (10–25 m), and the remainder were littoral (<10 m) stations. Two stations in each zone were used in May–October 1982. Some winter sampling was carried out in February 1983 and comparisons were made with other studies (Johnson and Brinkhurst 1971; Siegfried 1985) to define life history chronology. Macroinvertebrates were collected with an Ekman dredge (15.24 × 15.24 cm) and screen pail with 0.5-mm apertures. A finer screen (0.25 mm) was used to sieve extra duplicate samples in 1982 to assess extent of losses of young-of-year (YOY) *P. hoyi*.

Data on abundance of *P. hoyi* in earlier years were available from Cooper (1964) for 1959–62 and from J. Reckahn (unpubl. data) during 1972–73 and 1976–79 at Cooper's profundal stations. Reckahn and Cooper also used the large Ekman (15.24 × 15.24 cm) and sieves with 0.5- to 0.6-mm apertures. Techniques should be comparable among all studies. The most unbiased comparison of the data, including 1980–82 as well, was made by selecting August, September, and October data and stratifying these data into upper (32–37 m) and lower (45–55 m) profundal zones. By August, YOY are large enough to be sampled quantitatively by the conventional equipment used. Depth stratification of samples allowed examination of potential differences among studies attributable to depth.

To describe the method used to calculate production, a general description of the life history of *P. hoyi* in the profundal zone of South Bay follows. The time of occurrence of gravid and spent adults indicated that young were released in March and April, so April 1 was assumed to be the initial time of each cohort. Young are termed YOY until the end of the first calendar year, juveniles through the second year, and adults in January–March of the third year.

Lengths of *P. hoyi* (base of antenna to insertion of telson) were measured with a photographic enlarger. Images at 5× magnification were copied and measured with dividers. A series of subsamples of *P. hoyi*, each with specimens of approximately equal length, was measured for both length and dried weight (30°C). Data were applied to the model $W = aL^b$. From length data and the length–weight equation $\log_e W$(milligrams) =

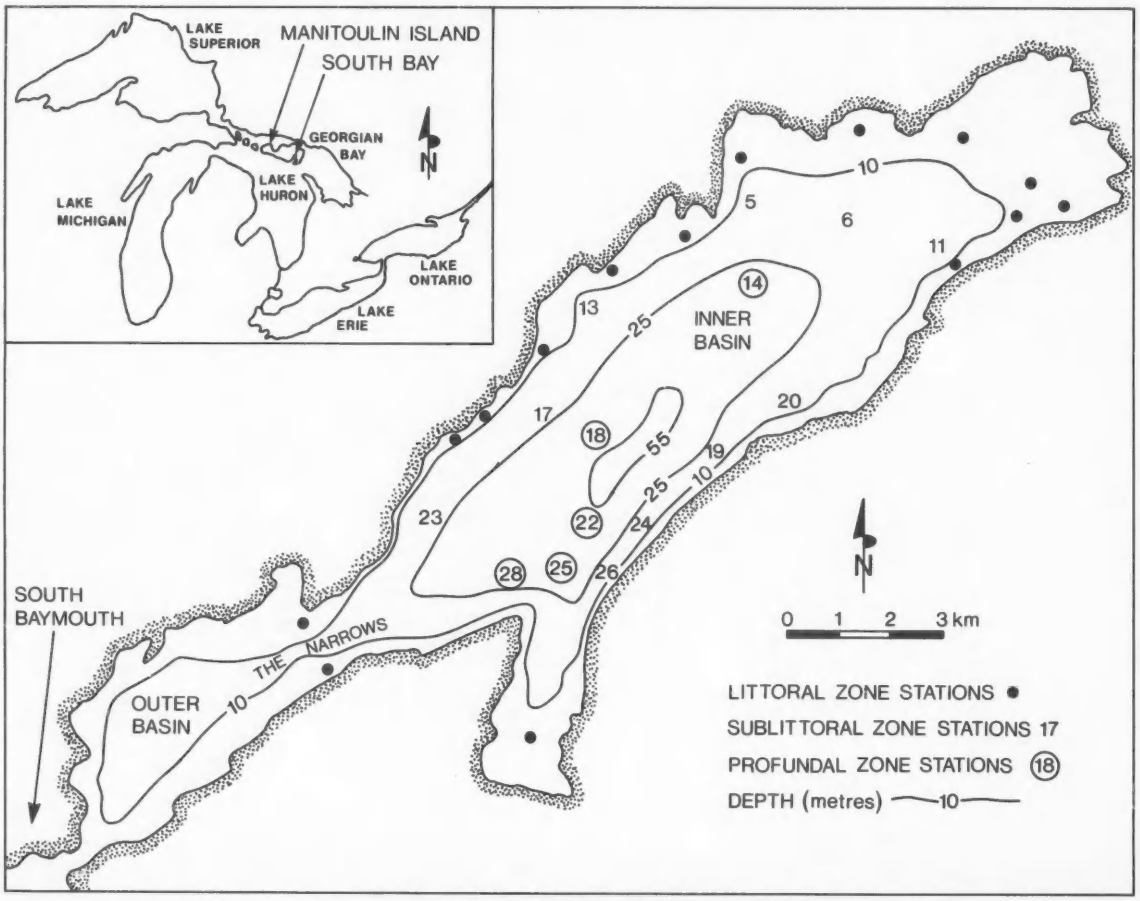

FIG. 1. Location of sampling stations in South Bay, Lake Huron.

$-6.889 + 3.404 \log L$(millimetres), weight of every individual was calculated and then the mean and confidence intervals ($p < 0.05$) were calculated for each cohort, month, and station where *P. hoyi* was present.

Production was calculated separately for sublittoral and profundal stations as mean biomass × instantaneous growth rate ($P = B \times G$ where P is production (grams per square metre per day), B is mean cohort biomass (grams per square metre), and instantaneous growth rate $G = (\log_e W_2 - \log_e W_1)/t$ with W in milligrams and t in days from the April initialization of each cohort). Mean individual weights through the 2-yr life of each cohort were fitted to two separate growth models to calculate values of G. YOY weights with time had lowest variance in a linear model, compared with other possibilities, while a von Bertalanffy curve was used for the juvenile phase. The asymptotic juvenile length was determined from a Walford regression, where a L_{t+1} vs. L_t regression has slope k and the y-axis intercept is $L^\infty (1-k)$, from which L^∞ is then calculated (Ricker 1975). Values of G for all time intervals were obtained using mean weights calculated from the weight–time regressions. The final YOY weight and the initial juvenile weight of each cohort were used to calculate G for the corresponding time interval.

The spreadsheet employed adequately describes production calculations. Columns were as follows: 1, date; 2, time (days) from April 1 initiation of a cohort; 3, interval (days); 4, number (per square metre); 5, mean individual weight (milligrams) calculated from regression of observed weights vs. time (column 2); 6, biomass (grams per square metre) as number × mean individual weight (milligrams) (column 4 × column 5 divided by 1000); 7, instantaneous growth rate G (per day) for each time interval (column 3) and corresponding beginning and final weights (column 5); 8, mean biomass B (grams per square metre) as the average of initial and final biomass (column 6) for each time interval; 9, production for interval (grams per square metre per day) as $B \times G$ (column 7 × column 8); 10, production (grams per square metre) during interval (column 9 × column 3).

Extrapolation of abundance and mean weight of juveniles as they approached maturity during the winter months was carried out as follows. A regression of numbers with time, from when YOY were collected quantitatively to the last estimates of juveniles, was projected forward through the winter months to the end of March to estimate populations of the 1980 and 1981 cohorts. Populations of the 1979 cohort were projected forward by assuming a survival rate equal to the mean of the 1980 and 1981 cohorts. Mean weights were obtained by projection of von Bertalanffy growth curves toward asymptotic weights.

To complete estimates of *P. hoyi* production for three calendar years, requisite data on the 1979 cohort were back-projected through 4 mo (January–April 1980) and, for the 1982 cohort, were forward-projected through 3 mo (October–December 1982). Population and mean weight of the 1979 cohort from January through March 1980 were interpolated between assumed values for October 1979 (means in October for the 1980, 1981, and 1982 cohorts) and measurements made in early May 1980. Forward projection of populations of YOY of the 1982 cohort into November and December was done by assuming survival comparable with that measured in the 1980 and 1981 cohorts.

Production was integrated firstly for each of the 1980 and 1981 cohorts annually and through their 2-yr duration and secondly as the sum of production of all cohorts present in each calendar year.

TABLE 1. Populations (m^{-2}) of *P. hoyi* in South Bay. Data for 1959–62 are from Cooper (1964), for 1972–79 from Reckahn (pers. comm.), and for 1980–82 from the present study.

	Profundal zone		
Year	Upper (32–37 m)	Lower (45–55 m)	Mean
1959	325	306	316
1960	—	77	77
1961	4690	—	4690
1962	2412	4000	3206
1972	1078	—	1078
1973	1608	—	1608
1976	947	2076	1512
1977	1174	1448	1311
1978	1531	2316	1924
1979	1812	2345	2079
1980	473	2330	1587
1981	653	3945	2490
1982	—	3481	3481
Mean	1519	2233	1951
SE$\bar{x}$	368	410	354

Results

Abundance and Distribution

Data on abundance of *P. hoyi* in the profundal zone of South Bay are available for 13 of 24 years during the 1959–82 period (Table 1). Abundance of *P. hoyi* in the upper and lower profundal zones varied from 325 to 4690·m^{-2} and from 77 to 4000·m^{-2}, respectively. Numbers were greater in the lower than the upper profundal in all years where data on both zones were available, except one, but the means were not significantly different. The mean population of *P. hoyi* in all profundal samples was 1951·m^{-2} with confidence limits of 1243–2659·m^{-2} ($p < 0.05$). Biomass of *P. hoyi*, converted from volume displacement measurements, varied from 1.4 g·m^{-2} in 1959 to 11.2 g·m^{-2} in 1961 (Cooper 1964). During the present study, biomass in samples averaged 6.3, 10.1, and 8.5 g·m^{-2} wet weight in 1980, 1981, 1982, respectively (J. Brookham, unpubl. data). Standing stocks in dry weight should be 15–20% of these values (M. G. Johnson, unpubl. data).

In 1980–82, *P. hoyi* abundance increased with depth, with an average population in all samples of 54·m^{-2} in the sublittoral zone and 2173·m^{-2} in the profundal zone. Only a small part of the population of each cohort examined occurred above the profundal zone (Fig. 2). No *P. hoyi* were collected in the littoral zone.

The distribution of juvenile *P. hoyi* was more limited than that of YOY, particularly during the summer months. YOY were present in some sublittoral zone collections at all 18 sampling times although their mean number seldom exceeded 100·m^{-2}. In contrast, juvenile *P. hoyi* were absent in sublittoral samples at 50% of the sampling times, usually July, August, and September collections. YOY *P. hoyi* were collected at sublittoral zone stations with temperatures of 9.0–21.3°C, while juvenile *P. hoyi* were collected at temperatures of 11°C and lower, that is, only at stations cooler than the mean temperature of the

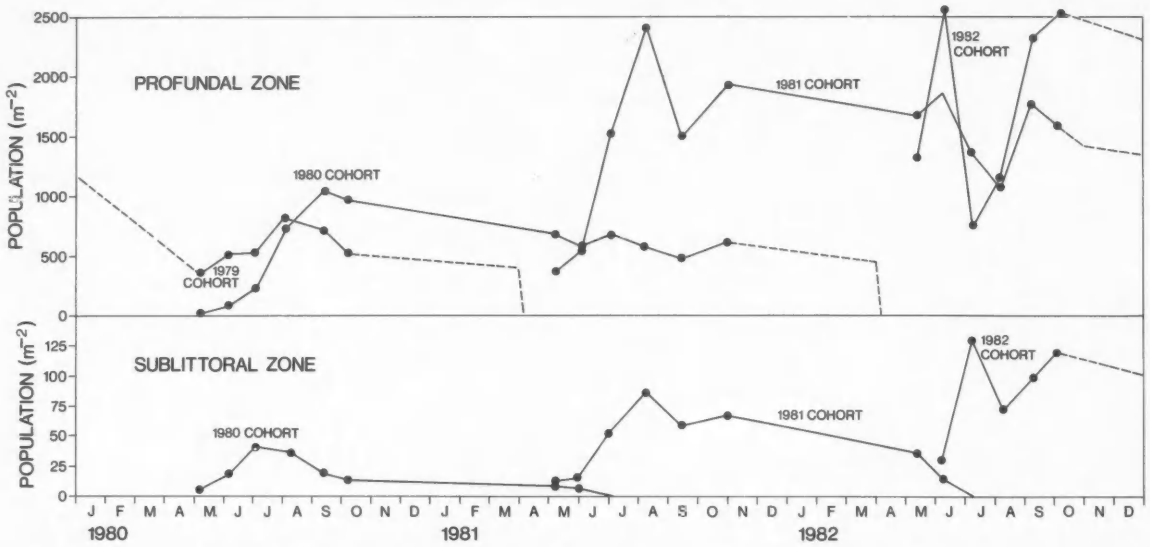
PROFUNDAL ZONE
POPULATION (m^{-2})
1979 COHORT
1980 COHORT
1981 COHORT
1982 COHORT
SUBLITTORAL ZONE
POPULATION (m^{-2})
1980 COHORT
1981 COHORT
1982 COHORT
J F M A M J J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D
1980
1981
1982

FIG. 2. Mean number of *P. hoyi* in profundal and sublittoral samples. Broken lines are extrapolated numbers, based on life history information and specific assumptions provided in the text.

sublittoral zone (12.5°C in July, 14.3°C in August, and 16.3°C in September compared with 7.1, 7.5, and 7.8°C in the profundal zone). Spent females were taken at all profundal stations in May and at some stations in June, but they were collected only in low numbers at four of the seven sublittoral stations at 14–20 m. Gravid females were collected only in May 1981 (in addition to qualitative winter collections), only from the profundal zone, and usually by a single specimen in one of the two replicates at each station.

Production

Growth rates

YOY and juveniles were easily separated on the basis of weight in all years. Significant differences in mean weight of the same cohort between stations were observed rarely among profundal stations and usually between sublittoral and profundal stations. Consequently, growth rates of *P. hoyi* in sublittoral and profundal zones were calculated separately. In growth equations, variance due to regression of mean weight versus time was high for YOY: 97, 98, and 93% for the 1980, 1981, and 1982 cohorts, respectively, in the profundal zone and 87, 93, and 82% for the three years in the sublittoral zone where fewer measurements were available. For juveniles the variance due to regression (using von Bertalanffy curves) was 77, 77, and 81% for the 1979, 1980, and 1981 cohorts, respectively.

YOY *P. hoyi* at sublittoral stations grew more rapidly than those from profundal stations (Fig. 3). Mean weights were similar in May and June in the two zones when mean temperatures differed by only 1–3°, but they diverged thereafter as the temperature differential increased. At the time of the October series, means calculated from regressions were significantly greater in the sublittoral zone (by 2 times in 1980, 1.6 times in 1981, and 2.7 times in 1982). Data on growth rate and abundance (Fig. 2) suggest that YOY born in the sublittoral zone remained there through their first year. The scarcity of juveniles in the following spring and absence in summer was the result of either low survival or movement of juveniles to the profundal zone.

Growth of YOY in the profundal zone was similar in the three years. G averaged 0.020, 0.019, and 0.017 in the May–October period in 1980, 1981, and 1982, although the mean weight on a given date was much more variable among years. Temperature was not significantly different among years. YOY abundance in 1981 and 1982 was at least twice the abundance in 1980, apparently with little effect on growth rate.

Collecting methods had the potential to distort growth rates of YOY in May and June when *P. hoyi* were small. Certainly their abundances then suggested incomplete sampling (Fig. 2). The number of YOY *P. hoyi* collected by the 0.25-mm sieve was 75% higher than the number collected in the 0.50-mm sieve. However, mean individual weight was almost identical in collections by the two sieves. The difference in mean weight was <10% in May and June. This result, together with the observation of regular, linear growth by YOY (Fig. 3), suggests that calculated growth rates were not biased by sampling methods.

The asymptotic growth model is appropriate for juvenile and maturing *P. hoyi* because weight of gravid animals in early spring is close to the weight of juveniles in the previous fall. In the Bay of Quinte, Johnson and Brinkhurst (1971) found similar absolute caloric content in nongravid animals collected in early fall and gravid animals taken in late winter and concluded that production of eggs and young was based on the equivalent of stored energy.

Migration of faster growing animals of the same cohort from the sublittoral zone to the profundal zone (which are of equal area in the inner basin of South Bay) would complicate the calculation of growth rate in juveniles only if the migrants were relatively abundant. In South Bay the greatest abundance of sublittoral juveniles in May was $36 \cdot m^{-2}$ in 1982 (Fig. 2). In 1980, juveniles were absent in samples from the sublittoral zone, and in 1981 they averaged only $9 \cdot m^{-2}$. Juvenile abundance in the profundal zone in May was never less than an order of magnitude greater than the highest abundance of juveniles in the sublittoral zone. Therefore, although there is no proof

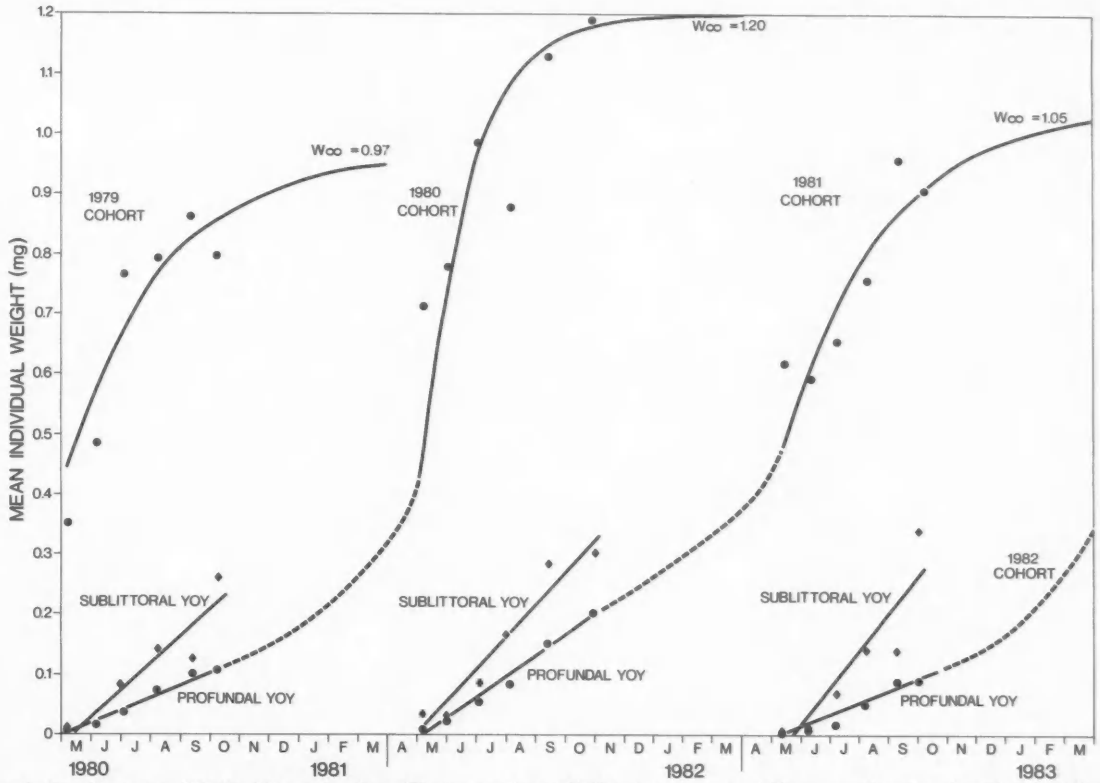

FIG. 3. Growth curves of *P. hoyi* for each cohort. The young-of-year (YOY) stage was one of linear growth and profundal and sublittoral populations were separate until juveniles migrated from the sublittoral to profundal zone. Juvenile and adult growth fit satisfactorily to the Walford model, adult growth through January–March being asymptotic toward W_∞.

that all surviving juveniles in the sublittoral zone migrated to the profundal zone as warming occurred, this would not significantly alter the estimate of growth rate of *P. hoyi* which had remained in the profundal zone since birth.

Growth (G) of juveniles in 1981 exceeded growth of juveniles in 1980 and 1982, and asymptotic individual animal weight was greater in 1981 (1.20 mg) than it was in 1980 (0.97 mg) and 1982 (1.05 mg). These differences are not large and they do not appear to be attributable to population sizes of juvenile *P. hoyi*, which were similar in 1980 and 1981.

The patterns of instantaneous growth rate (G) through time for each cohort were similar (Fig. 4). These were characterized by declining G through summer and fall of year 1, a springtime period of increased growth of juveniles (probably in response to increasing temperature and the spring bloom of phytoplankton and deposition of new organic matter), followed by a second period of decelerating growth toward the adult stage. The patterns are remarkably similar to those observed in the Bay of Quinte and adjacent waters of Lake Ontario (Johnson and Brinkhurst 1971, fig. 8). Values of G were higher initially in the sublittoral compared with the profundal zone (Fig. 4) but they showed the same temporal decline.

Biomass

The patterns in biomass per square metre (Fig. 4) were less similar among years than observed in patterns of G, with a large increase in 1982 of juveniles of the 1981 cohort. Mean biomass of juveniles in 1982 was 1.09 $g \cdot m^{-2}$ compared with 0.33 and 0.52 $g \cdot m^{-2}$ in 1980 and 1981, respectively. This difference is assumed to be partly an artifact of the sampling program in 1982, when only two of the five profundal stations were sampled. In 1980 and 1981, means of abundance of juvenile *P. hoyi* at these two stations (18 and 25) were 1.8 and 1.5 times the means based on all five profundal stations. However, at stations 18 and 25 alone, juvenile numbers in 1982 were 1.5 times those in 1980 and 1.7 times those in 1981. The increase in juvenile biomass in 1982 relative to other years probably was real but it was exaggerated. Overestimation of production of *P. hoyi* in 1982 and of the 1981 cohort likely occurred relative to 1980 and 1981 estimates.

The problem of underrepresentation of YOY in May, June, and July samples were examined in two ways. Comparison of numbers collected in coarse and fine sieves indicated a loss of about 40% of YOY biomass. Back-projection of numbers of *P. hoyi* in regressions of number versus time for each cohort (during the period it was sampled quantitatively) indicated a loss of about 10% in July, 23% in June, and 72% in May, that is, about 35% overall. The probable effect of this underrepresentation and the absence of sampling in April on estimates of production will be examined subsequently.

Net production

Temporal trends in net production of the 1980 and 1981 cohorts in the profundal zone consisted of four general phases:

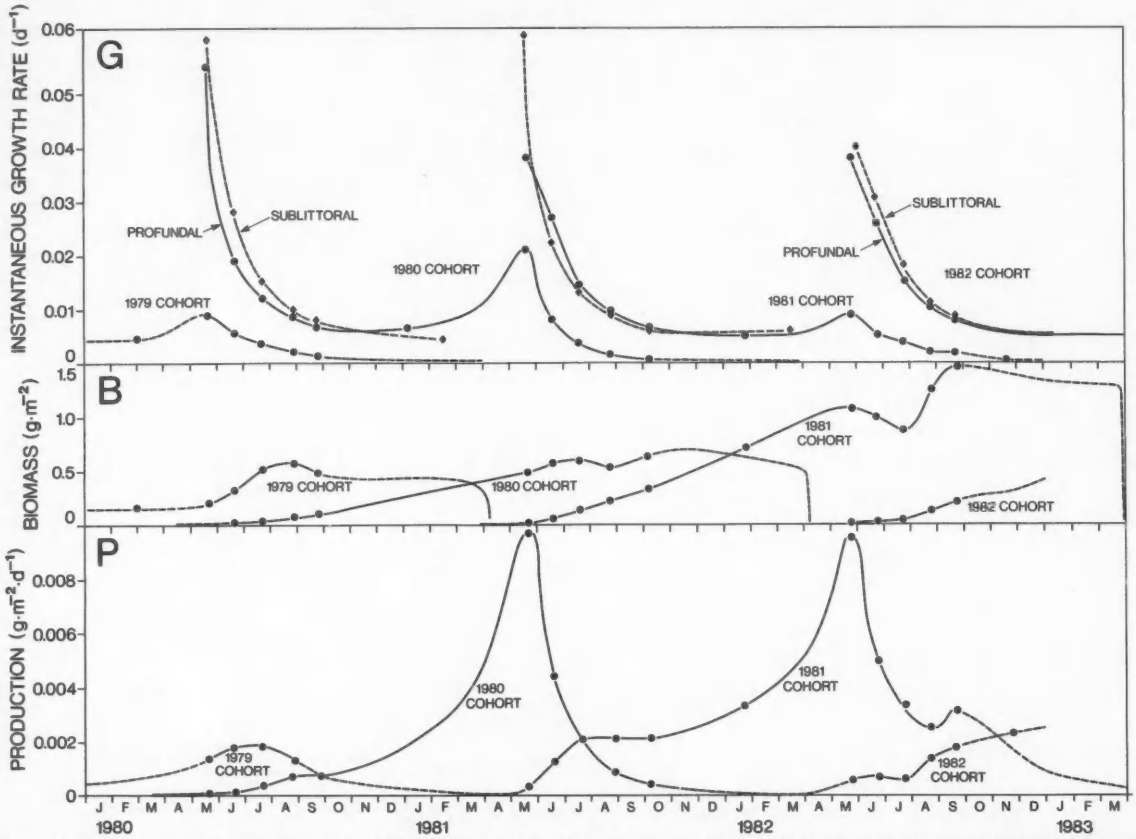

FIG. 4. Instantaneous growth rates (G), mean biomass (B), and production (P) of each cohort of *P. hoyi* in inner South Bay. Values of G were calculated from mean individual weights from weight–time regressions (Fig. 3) and between final YOY and initial juvenile weights. B is the product of population (m^{-2}) and the observed mean individual weights, and $P. = B \times G$. Growth rates of sublittoral YOY are shown here but their mean biomass and production are minimal and are not shown. Biomass and production shown here occurred in the profundal zone.

an acceleration of production through the first spring and early summer, a phase of slowly increasing production in late summer and fall, a sharp rise in production by juveniles during the second spring, and a phase of declining production toward maturity (Fig. 4).

This study provided estimates of production and *P/B* for the 1980 and 1981 cohorts separately and estimates for combined cohorts for two calendar years, 1981 and 1982 (Table 2). The estimate of production by *P. hoyi* in the profundal zone in 1980 did not include production of adults of the 1978 cohort. Data on 1980 and 1981 cohorts indicated that the estimate for calendar year 1980 probably was low by only 1–2%. In the profundal zone, approximately 25% of cohort production was by YOY, 74% by juveniles, and 1% by mature *P. hoyi* (Table 2). *P/B* values of 1.2–1.7·yr^{-1} were within the range of 1–2·yr^{-1} calculated by Johnson and Brinkhurst (1971) for *P. hoyi* in eastern Lake Ontario, with *P/B* increasing from deepwater Lake Ontario to the lower Bay of Quinte in relatively shallower and warmer sediments. Annual *P/B* averaged 1.1 in *P. hoyi* in Lake Michigan at 65–115 m (Lubner 1979), comparable with deepwater Lake Ontario. In a comparison of *P. hoyi* at shallow (15 m) and deep (42 m) sites in southeastern Lake Michigan, Winnell and White (1984) calculated mean *P/B* values of 1.27 and 3.13, respectively. *P/B* values calculated for the 1980 and 1981 cohorts in South Bay over their life span were 2.8 and 2.4, respectively. These are 2-yr values which translate to a mean annual *P/B* of 1.3·yr^{-1}. The probable overestimation of both biomass and production in 1982 relative to 1980 and 1981 should not have altered *P/B* values.

Production of *P. hoyi* in the sublittoral zone by the 1980 and 1981 cohorts was 0.012 and 0.054 g·m^{-2}, respectively, compared with 0.99 and 1.86 g·m^{-2} in the profundal zone. YOY contributed as much or more to cohort production than juveniles in the sublittoral zone because juveniles did not remain there. Adults were so scarce that their production and biomass would be insignificant. In the sublittoral zone, annual *P/B* values were 3.5·yr^{-1} in 1980, 2.8·yr^{-1} in 1981, and 3.0·yr^{-1} in 1982.

The significance of incomplete sampling of small YOY was examined. An estimate of YOY production in the profundal zone which occurred in April prior to sampling was obtained by back-projection of numbers and mean weights to April 1. The underestimate of production due to underrepresentation of YOY in spring samples was determined using these back-calculated numbers. Net production estimates corrected for these "losses" of YOY *P. hoyi* were greater than initial estimates by 19 and 10% for the 1980 and 1981 cohorts, respectively, through their first year, April–March inclusive. In terms of total

TABLE 2. Estimates of production ($g \cdot m^{-2}$ dry weight) of *P. hoyi* by cohort and calendar year in the main basin of South Bay. Life stages noted are young-of-year (Y), juvenile (J), and adult (A) as defined in the text. Production by adults in the sublittoral zone was not measured because of their scarcity. This omission will not affect the *P/B* values significantly.

	Cohort					
Year	1979	1980	1981	1982	Annual sum	*P/B* (yr^{-1})
Profundal zone						
1980	0.35 (J)	0.22 (Y)			0.57	1.4
1981	0.02 (A)	0.76 (J)	0.52 (Y)		1.30	1.7
1982		0.01 (A)	1.29 (J)	0.29 (Y)	1.59	1.2
1983			0.04 (A)			
Cohort sum				1.85		
P/B 2-yr		0.99		2.4		
		2.8				
Sublittoral zone						
1980	0 (J)	0.008 (Y)			0.008	3.5
1981	? (A)	0.004 (J)	0.026 (J)		0.030	2.8
1982		? (A)	0.028 (J)	0.038 (Y)	0.066	3.0
1983			? (A)			
Cohort sum		0.012	0.054			
P/B 2-yr		3.2	3.5			

production by the 1980 and 1981 cohorts, these "losses" were only 8 and 5%, respectively, of estimated production.

Discussion

The amphipod *P. hoyi* was consistently the dominant profundal benthic macroinvertebrate in samples collected in 13 yr in the main basin of South Bay, comprising typically 70–90% of all animals collected, with mean summer standing stocks of about $2100 \cdot m^{-2}$ and 8 $g \cdot m^{-2}$ wet weight (1.4 $g \cdot m^{-2}$ dry weight).

Production of *P. hoyi* occurred mainly in the profundal zone. Some *P. hoyi* adults moved to the sublittoral zone and released young there in late winter, but only YOY *P. hoyi* were collected in summer. Probably, juveniles left the sublittoral zone throughout the spring as warming occurred. The preference for cooler temperatures by juveniles compared with YOY was suggested by the presence of juveniles in the spring only at stations where temperature was <11°C, while YOY were more widely distributed in the sublittoral zone in relation to temperature. Movement of juvenile *P. hoyi* to cooler waters was reported by Dermott (pers. comm.) in Lake Memphremagog, Quebec, by Siegfried (1985) in Lake George, New York, and by Smith (1972) in Lake Superior. A second hypothesis is that predation by fish virtually eliminated the low juvenile populations in the sublittoral zone. Predation may influence numbers of *P. hoyi* temporally (Johnson and McNeil 1986) and, therefore, possibly could be a controlling factor spatially also. However, Cooper (1964) reported that *P. hoyi* completed its life cycle in 1 yr in the smaller outer basin of South Bay, which is shallow (13 m), weakly stratified, and relatively warm (13–15°C) over sublittoral sediments. Because *P. hoyi* maintained populations in the outer basin in the presence of an abundant fish community, the hypothesis of "preference" by *P. hoyi* for the cooler profundal zone has more support than the hypothesis of elimination by predation.

Two sources of bias in estimates of production were described. First, underrepresentation of YOY in spring samples did not distort growth rates but it led to underestimates of production directly proportional to underestimates of biomass. However, underestimation of production was <10% of cohort production. The second error was introduced by inadvertent selection in 1982 of two of five profundal stations which had greater than average abundance of juvenile *P. hoyi* in 1980 and 1981. Consequently, production of juveniles in 1982 probably was overestimated by 1.5–1.8 times relative to production in the other two years based on five stations. Production of all *P. hoyi* in 1982 probably was close to that in 1981 and remained greater than production in 1980.

The South Bay data provide further evidence that biomass reflects production consistent with a turnover rate (*P/B*) set by temperature (Johnson and Brinkhurst 1971). If turnover rate is affected primarily by temperature, then biomass must vary in relation to import of utilizable food. The combined Quinte and South Bay data support this hypothesis. The relationship between *P/B* and temperature was consistent, even though production of *P. hoyi* at Quinte was 6 times production in South Bay. Results from Lake Michigan appear to be similar although *P/B* was compared with depth rather than temperature (Winnell and White 1984). *Pontoporeia hoyi* at the "Lake Ontario" station had a mean *P/B* of $2.0 \cdot yr^{-1}$ at a mean annual temperature of 6°C. At the "Conway" station, *P/B* averaged $2.3 \cdot yr^{-1}$ at 7°C. *Pontoporeia hoyi* from the profundal zone of South Bay had a mean *P/B* of $1.4 \cdot yr^{-1}$ at a mean annual temperature of 4.7°C and, in the sublittoral zone, a mean *P/B* of $3.1 \cdot yr^{-1}$ at 7.5°C. In regression analysis of these data, temperature accounted for 90% of the variability in turnover rate. Turnover rates of 1, 2, and $3 \cdot yr^{-1}$ are predicted at mean annual temperatures of approximately 4, 6, and 8°C, respectively. Mean biomass of *P. hoyi* in South Bay during 1980–82 was 0.8 $g \cdot m^{-2}$ and mean biomass accompanying the Quinte production measurements in 1967–68 was 5 $g \cdot m^{-2}$. Integral primary production at station 22 in South Bay in 1982 and 1983 (30 occasions in May–September) averaged 53 mg $C \cdot m^{-2} \cdot h^{-1}$ (M. G. Johnson, unpubl. data). Integral primary production measurements during May–October in 1972–77 in the lower Bay of Quinte aver-

aged 210 mg $C \cdot m^{-2} \cdot h^{-1}$ (Millard and Johnson 1986). Relative differences in *P. hoyi* biomass and primary production are similar. No data on primary production in South Bay are available for 1980 and 1981 which would have permitted a comparison of changes in biomass and production of *P. hoyi* with changes in food supply. In Lake Michigan, Lubner (1979) reported a probable relationship between primary production and *P. hoyi* production, reflected moreso by changes in biomass than by individual weight.

An index using both mean annual biomass and temperature may provide useful estimates of production in benthic macroinvertebrate communities or by selected species. The predicting equation for macroinvertebrate community production in the Quinte study was $P = B(T^2/10)$ (Johnson and Brinkhurst 1971). Because of the dominance of *P. hoyi* in profundal benthos of much of the Great Lakes and its importance in diets of fish, a production index for *P. hoyi* would be useful in trophodynamic studies and of interest to researchers attempting to estimate potential fish production. More work is needed in this area because data on benthic macroinvertebrate numbers and biomass will continue to be generated, while production estimates likely will remain scarce.

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Acute Toxicity of Binary Mixtures of Five Cations (Cu^{2+}, Cd^{2+}, Zn^{2+}, Mg^{2+}, and K^{+}) to the Freshwater Amphipod *Gammarus lacustris* (Sars): Alternative Descriptive Models

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The combined results of 10 acute toxicity experiments, each testing the joint toxicity of two of the ions Cu^{2+}, Cd^{2+}, Zn^{2+}, Mg^{2+}, and K^{+}, were examined in terms of different response surface models which could be used to make decisions about limiting toxic components in mixtures. The classical probit model for simple similar action described experimental results satisfactorily with a model R^2 of 0.282; equations in which probit(*p*) was described directly by a linear combination of toxicant concentrations fit data significantly better, with an R^2 of 0.527. Equations with more complex linear terms and appropriate weighting factors applied to the residual sums of squares yielded R^2 values up to 0.931. Predicted LC50 values were midrange compared with published values. Based on the linear description of the probit response, K or Mg in combination with either of Cu, Cd, or Zn had additive effects, the combinations Cu and Cd, Cu and Zn, and Cd and Zn had more-than-additive effects, and Mg and K had less-than-additive effects. The relationships between the response surfaces, other described modes of joint action, the toxic units model, and mixture toxicity indices are discussed.

L'auteur a analysé les résultats de dix essais de toxicité aiguë comportant chacun un test de la toxicité mixte de paire d'ions Cu^{+2}, Cd^{+2}, Zn^{+2}, Mg^{+2} ou K^{+}, dans le contexte des diverses réponses de modèles de surface pouvant servir à décider des composantes toxiques limitantes des mélanges. Le modèle classique par probit s'appliquant aux effets simples du même type permettait de décrire les résultats expérimentaux de façon satisfaisante avec une valeur de R^2 de 0,282. Les équations pour lesquelles le probit (*p*) était directement décrit par une combinaison linéaire des concentrations de toxiques présentaient un ajustement aux données significativement supérieur, la valeur de R^2 étant de 0,527. Des équations comportant des termes linéaires plus complexes et des facteurs de pondération appropriés appliqués aux sommes des carrés résiduelles permettaient d'obtenir des valeurs de R^2 pouvant atteindre 0,931. Les valeurs de CL50 prévues se situaient au centre de la gamme des valeurs publiées. Il est apparu, sur la base de la description linéaire de la réponse du probit, que les ions K ou Mg agissant avec les ions Cu, Cd ou Zn présentaient des effets additifs, que les combinaisons Cu et Cd, Cu et Zn et Cd et Zn avaient des effets plus qu'additifs, tandis que la combinaison Mg et K avait des effets inférieurs à des effets additifs. L'auteur traite aussi des relations entre les surfaces de réponse, d'autres modes d'action conjointe, du modèle des unités toxiques et des indices de toxicité des mélanges.

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The effects of mixtures of toxicants in aquatic systems is a topic which has received renewed attention because of numerous cases of aquatic systems stressed by toxicants from many sources. In spite of the high level of concern and discussion about approaches to mixtures (Anderson and Weber 1975; EIFAC 1980, 1987; International Joint Commission 1981; Stich et al. 1982; Vouk et al. 1987), there are few scientifically defensible methods for making decisions about limiting toxic components. Decisions could be based on the results of acute toxicity tests, for example with the use of "application factors" (Kenaga 1982) which convert acutely lethal levels to tolerable ones for certain groups of toxicants. The ability to make such decisions would then depend on the existence of a model that describes the effects of all toxicants in all possible concentration combinations.

This study was initially intended to show which one of several models, often discussed in the literature as if they were alternative hypotheses, would best describe mortality in experiments with mixtures of toxicants. For example, it was expected that "simple similar action" (Bliss 1939), also called the concentration–addition model (Anderson and Weber 1975; EIFAC 1987), might best describe the results of experiments with copper and cadmium, two metals expected to act similarly. It was expected that "independent joint action," also called the response–addition model (Anderson and Weber 1975; EIFAC 1987), "independent action," or a mode of joint action inter-

mediate between the two (Bliss 1939), would best describe the results of experiments with copper and potassium, two ions expected to act differently. Then, it was hoped that the fusion of the two or expansion of one of two models might describe the results of more complex or combined experiments.

However, initial analyses of results from experiments with binary mixtures showed that simple similar action and independent joint action described the results of any given experiment equally well. At first this was assumed to be due to relatively large experimental errors that did not allow discrimination between models. However, closer scrutiny of the two models and their modifications (de March 1987b) showed that they were geometrically similar even if not algebraically identical. In practice the models were not alternative hypotheses, but alternative descriptive methods. Thus, it was decided that models which were based on the laws of probability, namely "independent joint action" and its extensions, would not be examined further. I believed that existing modifications of the basic definition were awkward. Also, I saw no reason why simple laws of probability should apply to whole organisms responding to stressors acting at several sites of action. Two other models, the classical probit model for simple similar action and its extensions, and various forms of linear models for describing the probit response, were examined further for their applicability to experimental results.

The extension of the classical probit response model (Bliss 1939) describing the noninteractive effects of several toxicants is as follows:

$$(1)\quad \text{Probit}(p) = k\cdot\ln(a_1\cdot C_1 e_1 + a_2\cdot C_2 e_2 + \ldots + a_n\cdot C_n e_n)$$

in which p is the percentage or probability of response, probit(p) is the probit transformation or normal equivalent deviate of p, a_1 to a_n are numerical coefficients, e_1 to e_n are exponents, and C_1 to C_n are concentrations of toxicants 1 to n. In the simple univariate model describing the effects of one toxicant, a lognormal distribution of the tolerances in relation to toxicant concentration is assumed; in a univariate but multivariable model such as the above, a lognormal distribution in response to a linear sum of concentrations is assumed. The chosen linear sum is partly based on kinetic and biochemical relationships between toxicants (Bliss 1939; Hewlett and Plackett 1952, 1959, 1964; Plackett and Hewlett 1952, 1963, 1967; Ashford and Smith 1964, 1965; Finney 1971). If all e_i's are equal to 1, then "simple similar action," the case in which the effects of any toxicant can be substituted by a multiple of the effects of any other, is described (Ashford and Smith 1964). If the e_i's are not equal, then nonparallel response curves describe the responses to individual toxicants.

Results of multivariable acute toxicity tests are also often described by statistical linear models such as regression analysis, mixed models, or by response surface methods (EIFAC 1987). The probability of response or a convenient transformation of it, such as probit(p), is described directly by linear combinations of functions of toxicant concentrations. This type of model is also examined in this paper because of its desirable statistical and mathematical properties.

The five ions used in these experiments, Cu^{2+}, Cd^{2+}, Zn^{2+}, Mg^{2+}, and K^+ were chosen because of sufficient knowledge of their physiological modes of action that different types of joint effects were expected. Balanced multifactorial designs for examining joint effects of toxicants are necessarily large and awkward, and some sort of experimental confounding or blocking is usually required to make experiments manageable. In this study, mixtures of only two toxicants were tested, with the assumption that third-order factor interactions would not be important or that the experimental error would be too large to examine higher order interactions. Experiments with only two toxicants were performed sequentially, with one toxicant from each experiment used in the next. This increased the probability of choosing an effective range of concentrations and also spread the use of each toxicant out over time. Also, toxicants were tested at different concentrations and at different ratios to ensure that response surface methodology could be used with the results.

TABLE 1. Summary information about the ten experiments performed.

Date (d/mo/yr)	Number of test cells	Toxicants and range of concentrations tested ($mg\cdot L^{-1}$)				
		Cu	Cd	Zn	K	Mg
02/09/83	21	0.013	0	0	4.24	0
		0.081			35.2	
02/10/83	22	0.027	0.005	0	0	0
		0.127	0.068			
12/10/83	23	0	0.010	0.024	0	0
			0.054	0.433		
22/11/83	24	0	0	0.043	0	2.63
				1.1		17.8
03/01/84	21	0	0	0	2.53	1.84
					10.8	12.2
16/01/84	22	0	0.002	0	4.46	0
			0.406		39.0	
25/01/84	23	0	0.017	0	0	4.27
			0.110			19.0
03/02/84	24	0.054	0	0	0	4.65
		0.392				43.2
10/02/84	24	0.155	0	0.410	0	0
		0.388		1.70		
23/02/84	23	0	0	0.066	12.93	0
				0.233	36.91	

Methods

Ten experiments, each examining the acute lethal response of the freshwater amphipod *Gammarus lacustris* (Sars) to mixtures with two of the five ions Cu^{2+}, Cd^{2+}, Zn^{2+}, Mg^{2+}, and K^+, were performed over 16 mo between October 1983 and March 1984 (Table 1). Each experiment consisted of 12–14 test cells with combinations of two toxicants in different ratios, 6 test cells with either of the two toxicants, and 2–4 control test cells with no toxicants. Experiments were done in a flow-through dispensing system using dechlorinated Winnipeg tap water, with the following average chemical mesurements: pH 7.8, hardness 80 mg $CaCO_3\cdot L^{-1}$, conductivity 200 $\mu S\cdot cm^{-1}$, 720 μmol dissolved inorganic $C\cdot L^{-1}$, 900 μmol dissolved organic $C\cdot L^{-1}$, 1.16 mg $K^+\cdot L^{-1}$, 5.84 mg $Mg^{2+}\cdot L^{-1}$, 6 mg $Cl^-\cdot L^{-1}$, and 7.7 mg $SO_4^{2-}\cdot L^{-1}$. Water was renewed completely every 2 h in each of the twenty-one to twenty-four 500-mL test cells, with a mean of 27 ± 8 animals per test cell. Most experiments lasted 6 d, one (February 23, 1984) lasted 3 d because complete mortality was achieved in all test cells by this time. Concentrations were calculated by measuring the dilution rate in each test cell before and after each experiment and occasionally confirmed analytically by the Metals Analysis group at this Institution. Additional acute toxicity tests with NaCl and $NaSO_4$ showed that the anion levels and salinities in these experiments were not at all stressful to *G. lacustris*.

Gammarus lacustris were obtained in the late fall from Nora Lake near Erickson, Manitoba. They were kept in the laboratory at cold temperatures near 5°C and at constant illumination so that reproduction would not be induced (de March 1982). Occasional accidental warm spells near 20°C aged animals rapidly, and consequently, control mortalities increased during 1984. In each experiment, dead animals were counted and removed at least every 8 h, and more often if rapid mortality occurred. Dead animals were easily distinguished in mixtures with metals because they quickly became turgid. Animals exposed to only K^+ or Mg^{2+} often had to be prodded for a reaction to distinguish signs of life.

In each test cell the acute mortality response over time was usually an S-shaped curve. The response in each test cell was described by the following curvilinear equation:

$$(2)\quad \text{Probit}(p) = a{\cdot}T^2 + b{\cdot}T + c + \epsilon$$

in which probit(p) is the probit of the cumulative percent mortality, T is the elapsed time in hours, a, b, and c are fitted coefficients, and ϵ is the residual error in the model. The fit of data to Equation (2) for each test cell was excellent. The error around these fitted equations were ignored because they constituted the subplot error in a repeated measures design (Cole and Grizzle 1966), which does not contribute to the evaluation of the joint effects of the toxicants. Further statistical analyses were performed with two summary statistics from these curves, the expected probit responses at 48 h (probit(p48)) and/or at 96 h (probit(p96)). The 96-h mortality at various toxicant concentrations in three of 10 experiments is shown in Fig. 1.

All statistical analyses, mainly curve fitting, were performed with the SAS Statistical Package (SAS Institute Inc. 1982). The procedures used to fit the data to the model equations were PROC NLIN (nonlinear regression procedure) and PROC GLM (general linear models procedure). PROC NLIN prints only R^2 values (multiple correlation coefficients) based on sums of squares not corrected for the mean. Corrected R^2 values were found by calculating 1 − (sums of squares for error)/(total sums of squares) so that the R^2 values for all models could be compared.

Results

In 9 of these 10 experiments analysed individually, either p96 or probit(p96) was significantly correlated with levels of both toxicants ($P \leq 0.05$). The exception was the Cd–Mg experiment in which only Cd correlated with the response. The R^2 values obtained by fitting the equation probit(p96) and/or probit(p48) $= a{\cdot}C_1 + b{\cdot}C_1^2 + c{\cdot}C_2 + d{\cdot}C_2^2 + k$ for each experiment show that responses were moderately predictable within experiments (Table 2). The 96-h measurements were believed to be more reliable than those at 48 h, since toxicant concentrations were chosen to give a range of responses at 96 h.

A complete range of possible responses was not obtained in each experiment (Table 2). However, since each toxicant was used in four experiments, a reasonable range of responses was obtained for all toxicants except Mg. Correlation coefficients between probit responses and toxicant concentrations utilizing data from all 10 experiments combined were often significant, but expectedly low because each was calculated over a range of levels of the second toxicant in the mixture (Table 3).

Classical Probit Response Models

Fitting the 96-h probit responses (probit(p96)) to Equation (1) yielded a response curve (not given) described with an R^2 of 0.310. Responses in control test cells (when all of C_1 to C_5 were zero) could not be used in fitting this curve; thus, this equation was fitted with only 170 of 204 points. Forcing a fit of simple similar action (that is, forcing all e_i's to equal 1) yielded an R^2 of 0.282. The overall fit of both models was significant ($P \leq 0.05$), but individual fitted parameters were not all so. The first model accounted for a significantly larger sum of squares than the latter at the $P \approx 0.20$ level using the generalized likelihood ratio test (Graybill 1976). Thus it is likely that some of the exponents were significantly different from 1 or from each other in this model, and simple similar action would not describe the joint effects of all pairs of toxicants. Even with this lack of significance, predicted LC50 values for all toxicants were within ranges reported in the literature, which often vary by an order of magnitude (Sprague 1985).

Textbooks on probit analysis suggest fitting probit curves with a weighting factor inversely proportional to the estimated variance applied to the residual sums of squares (Finney 1971; Hubert 1984). In fitting the above curves once more, such weighting was done, and the R^2 values increased to 0.795 and 0.715 for the above two equations, respectively. However, predicted LC50's and relative toxicities from the equations were orders of magnitude different from reported values. This may be due to the weighting factor emphasizing several high and slightly outlying responses, thus twisting the whole response

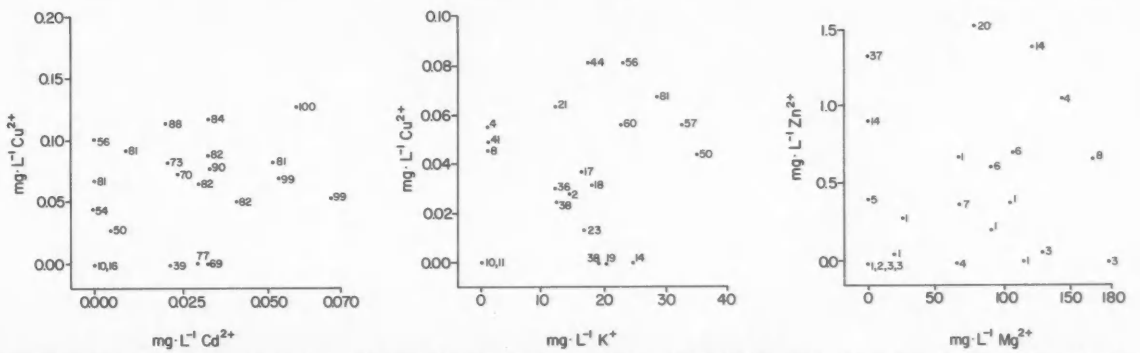

FIG. 1. Results of 3 of 10 experiments done with binary mixtures. The predicted mortality at 96 h, obtained from Equation (2), is plotted against the concentrations of the two toxicants.

TABLE 2. Predictability of mortality response in individual experiments. R^2 for individual experiments were obtained by regressing probit($p48$) and probit($p96$), from Equation (2), against linear and quadratic concentration terms of toxicant concentrations, that is probit(p) = $a \cdot C_1 + b \cdot C_2 + c \cdot C_1^2 + d \cdot C_2^2 + k$. Overall fit significant at $*P \leq 0.10$ and $**P \leq 0.05$.

Date (d/mo/yr)	Dependent variable	Toxicants	R^2	n	Response range (% mortality)
02/09/83	Probit($p48$)	Cu, K	0.363*	21	0–40
	Probit($p96$)		0.541**		2–81
02/10/83	Probit($p48$)	Cu, Cd	0.755**	22	6–94
	Probit($p96$)		0.824**		6–100
12/10/83	Probit($p48$)	Cd, Zn	0.460**	23	7–60
	Probit($p96$)		0.792**		7–87
22/11/83	Probit($p48$)	Zn, Mg	0.026	24	0–97
	Probit($p96$)		0.672**		1–97
03/01/84	Probit($p48$)	Mg, K	0.706**	21	0–22
	Probit($p96$)		0.682**		5–45
16/01/84	Probit($p48$)	K, Cd	0.281	22	0–16
	Probit($p96$)		0.511**		1–60
25/01/84	Probit($p48$)	Cd, Mg	0.282	23	0–7
	Probit($p96$)		0.337		2–19
03/02/84	Probit($p48$)	Mg, Cu	0.598**	24	0–22
	Probit($p96$)		0.712**		4–43
10/02/84	Probit($p48$)	Cu, Zn	0.460**	24	6–82
	Probit($p96$)		0.887**		21–94
23/02/84	Probit($p48$)	Zn, K	0.873**	23	20–99

TABLE 3. Simple correlation coefficients between probit ($p48$) or probit($p96$), from Equation (2), and concentrations ($mg \cdot L^{-1}$) of individual toxicants in 10 experiments combined. The number of test cells of a possible 227 is in parentheses. Correlations significant at $*P \leq 0.10$ and $**P \leq 0.05$.

	Correlation with toxicant				
Dependent variable	Cu	Cd	Zn	K	Mg
			Results from all test cells		
Probit($p96$)	0.452**	0.154**	0.255**	0.045	−0.219**
	(227)	(227)	(227)	(227)	(227)
Probit($p48$)	0.224**	−0.033	0.127	0.374**	−0.186**
	(204)	(204)	(204)	(204)	(204)
			Test cells with two toxicants excluded		
Probit($p96$)	0.512**	0.521**	0.573**	0.311	0.409**
	(46)	(46)	(42)	(42)	(44)
Probit($p48$)	0.175	0.209	0.064	0.228	0.523**
	(50)	(50)	(49)	(47)	(51)
			Test cells with no toxicant also excluded		
Probit($p96$)	0.263	0.339	0.710	0.651	0.285
	(12)	(12)	(8)	(9)	(10)
Probit($p48$)	0.143	0.309	0.760**	−0.581	0.313
	(12)	(12)	(11)	(9)	(12)

surface. Plots of observed versus predicted values in the next section will confirm that the probit transformation alone stabilized variances sufficiently such that the weighting factor was not required.

Linear Models

The fitting of the same 170 probit($p96$) values to simple linear combination of concentrations, that is, to an equation containing only the first six terms of the model

$$(3)\quad \text{Probit}(p96) = k + a \cdot C_1 + b \cdot C_2 + c \cdot C_3 + d \cdot C_4 + e \cdot C_5 + f \cdot C_1^2 + g \cdot C_2^2 + h \cdot C_3^2 + i \cdot C_4^2 + j \cdot C_5^2 + l \cdot C_1 \cdot C_2 + m \cdot C_1 \cdot C_3 + \ldots \text{all cross-products} \ldots + \epsilon,$$

yielded an R^2 of 0.373. All regression coefficients were significantly larger than zero ($P \leq 0.05$). Fitting the first 11 terms of Equation (3) yielded an R^2 of 0.527. Adding the cross-products increased the R^2 value to 0.709. The increase in the sums of squares accounted for was significant in both cases ($P \leq$

0.05, generalized likelihood ratio test; Graybill 1976). The fitting of all 204 data points, as opposed to the 170 noncontrol responses, yielded an R^2 of 0.700. In all cases, the resultant equations predicted midrange LC50's when compared with the literature. The weighting factor applied to the three linear equations above yielded R^2 values of 0.800, 0.874, and 0.931, respectively. This time, LC50 predictions were reasonable.

Reduced Descriptive Equations

The chosen linear models obviously described experimental results better than the probit model with the same number of terms. To reduce the unnecessary complexity of the fitted Equation (3), terms for which the coefficients which were not significant at the $P \leq 0.005$ level were dropped in a stepwise manner until only terms significant at that level remained. The final descriptive equation was

$$\begin{aligned}(4)\quad \text{Probit}(p96) = {} & 5.731\cdot Cu + 22.75\cdot Cd + 221.7\cdot Cd^2 \\ & + 0.5431\cdot Zn + 0.02286\cdot K \\ & - 0.02386\cdot Mg + 494.2\cdot Cu\cdot Cd \\ & + 101.2\cdot Cd\cdot Zn + 0.01350\cdot K\cdot Mg \\ & + 3.783 \qquad R^2 = 0.639,\ n = 204.\end{aligned}$$

The mean control response predicted by the intercept is 11.18% = probit^{-1} (3.783), close to the actual control mortality of 12.2%. The 96-h LC50 concentrations for individual toxicants, calculated by sustituting probit(0.5) = 5.0 and by setting the concentrations values of other toxicants to zero, are 0.212 mg $Cu\cdot L^{-1}$, 0.0401 mg $Cd\cdot L^{-1}$, 2.24 mg $Zn\cdot L^{-1}$, and 53.2 mg $K\cdot L^{-1}$, respectively. These are midrange values in relation to published values (Clarke 1974). The binary combinations (Cu and Cd) and (Cd and Zn) were predicted to have more-than-additive effects on the probit response. Mg slightly decreased the toxicity of all toxicants except K. From the above equation, it is expected that 1 unit of Mg would reduce the LC50 of Cu by 0.004 unit, of Cd by 0.001 unit, and of Zn by 0.0435 unit. Mg is predicted to increase the toxicity of K. The standard error of the predicted response of the model was 0.621 probit unit, which would be approximately 23% near the 50% response and 20% near the 25 and 75% responses.

Plots of observed versus predicted probit values obtained from Equation (4) (Fig. 2A) suggest that the probit transformation was an appropriate variance-stabilizing one, with the residuals approximately normally distributed about the expected values.

It is possible to partly eliminate the experimental confounding by calculating an intercept for each experiment. This was done by using "Date" or "Experiment" as a class variable and concentrations as covariates in the SAS GLM procedure (SAS Institute Inc. 1982). The statistical analysis of the data is then an analysis of covariance, with the concentrations of the five toxicants as covariates. This analysis was done and, as before, terms not significant at the $P \leq 0.005$ level were dropped. The resultant descriptive equation was

$$\begin{aligned}(5)\quad \text{Probit}(p96) = {} & \text{Correction factor by experiment} \\ & + 2.552\cdot Cu + 7.910\cdot Cd + 0.03501\cdot K \\ & + 0.8289\cdot Zn + 331.1\cdot Cu\cdot Cd \\ & + 55.17\cdot Cd\cdot Zn \qquad R^2 = 0.818,\ n = 204.\end{aligned}$$

The predicted correction factor ranged from 2.880 = probit(1.7%) for the Zn–Mg experiment to 4.856 = probit(44.3%) for the Cu–Cd experiment. This correction factor is partly due to control mortality and partly to each experimental mean and is therefore not necessarily identical to the observed control mortality. The mean correction factor was 3.8736 = probit(9.4%). Predicted 96-h LC50 concentrations were 0.441 mg $Cu\cdot L^{-1}$, 0.126 mg $Cd\cdot L^{-1}$, 32.2 mg $K\cdot L^{-1}$, and 1.36 mg $Zn\cdot L^{-1}$. Mg was predicted to not have an effect. All four experiments with Mg (Table 1) had low control mortalities and low but not highly predictable response ranges which are largely accounted for by the correction factor. The standard error of the predicted response in this model was 0.448 probit unit, which is approximately 17% near the 50% response and 14% near the 25 and 75% responses. Observed versus predicted responses are shown in Fig. 2B and also show an acceptable distribution of error variances.

The response curve describing the 227 probit($p48$) values, obtained in the same manner, was

$$\begin{aligned}(6)\quad \text{Probit}(p48) = {} & \text{Correction factor for date} \\ & + 0.04327\cdot K + 0.02224\cdot Mg \\ & + 329.3\%Cu\cdot Cd + 3.591\cdot Cu\cdot Zn \\ & \qquad R^2 = 0.754,\ n = 227.\end{aligned}$$

The mean correction factor was 3.5616 = probit(7.5%). The predicted 48-h LC50 of K was 33.2 mg $K\cdot L^{-1}$, not notably different from the 96-h LC50 of 32.2 mg $K\cdot L^{-1}$. This confirmed the visual observation that K exerted its effects quickly or not at all. Mg also had slight toxic effects during this short period of time. The extrapolated 48-h LC50 for Mg was 64.7 mg $Mg\cdot L^{-1}$, a value with little credibility. Cu, Cd, and Zn were effective only in the presence of other metal ions, as described by the cross-products. It is reasonable that in this short period of time, some toxicants were less effective, or perhaps not effective at all, unless another was present. The standard error of this model was 0.5394 probit unit, approximately 20% near the 50% response.

The responses at 96 and 48 h are obviously correlated, since both were derived from the same temporal response curves. The correlation between probit($p96$) and probit($p48$) was 0.839. Equation (6) does, however, describe several features expected of a short-term response.

Discussion

The unexplained variance in these data is high and is known to be due to variation in dilution rates. Nevertheless, examination of the literature suggests that the lack of predictability may not be unusual, but may be conspicuous due to the experimental design and data presentation. In experiments performed in a traditional dilutor, the probability of obtaining a smooth monotonic response curve is high, since errors in estimating concentrations in neighboring test cells might be in the same direction. In these experiments the errors in calculating the concentrations were independent of each other in all test cells, increasing the probability of scatter in both directions. Also, the presented experiments were done within small concentration ranges chosen after performing preliminary tests or after examining the results of the last experiment. Correlations between the response and concentrations cannot be expected to be as obvious as in experiments with complete response ranges and toxicants used in geometrically increasing concentrations. The standard errors of the probit response suggest that fiducial limits of all toxicants used would be reasonable considering variation possible in replicate tests (Sprague 1985).

The fact that the linear models had a considerably better predictive ability than the traditional probit equations was surpris-

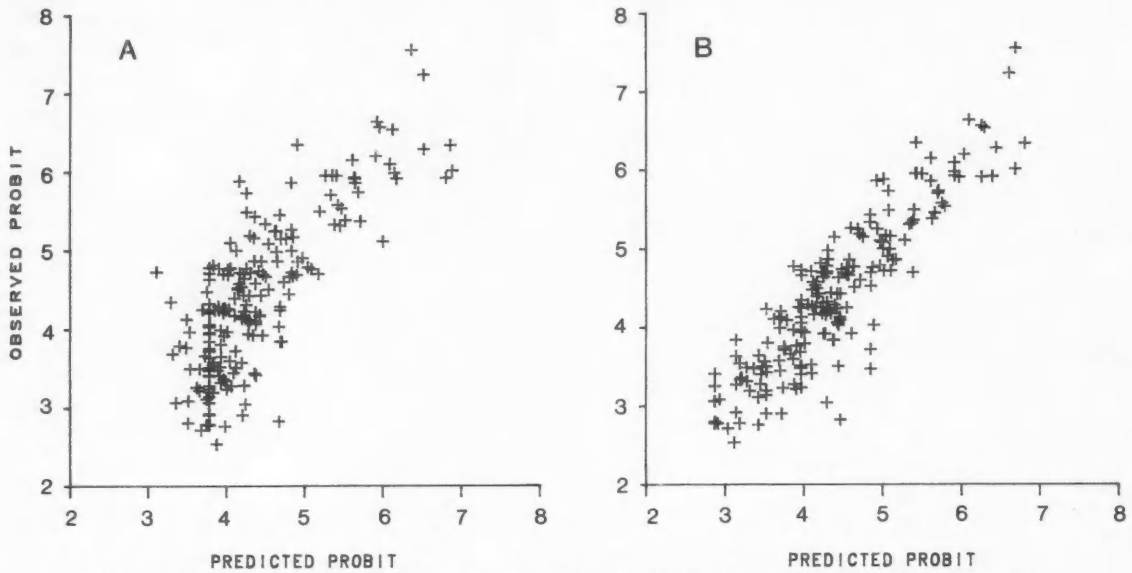

FIG. 2. Plots of observed versus expected probit values for data described by (A) Equation (4) and (B) Equation (5).

ing. The concept of the lognormal response to the linear combination of concentrations has seldom been questioned. The water used for these experiments did not have a strong buffering capacity; it may be that the responses were actually more normally than lognormally distributed over the toxicant concentrations used. Among the linear models, the ones with the correction factors by experiment are most likely the most desirable ones for both theoretical and biological reasons. Control mortalities varied between experiments; thus, a correction is appropriate. The response to each toxicant was still determined from four experiments spread out over time. It can be argued that the classical probit model also has a similar correction factor, since one of the main functions of the coefficients (a_i's in Equation (1) is to describe the asymptotic or control response.

There is an important conceptual difference between the linear and the classical probit models. In the linear model, a known and fixed form of the response, in this case probit(p), is partitioned into additive components. In the classical probit model, the response that is partitioned into additive components is EXP(probit(p)/k) (from Equation (1)), in which k can be determined only after the equation is fitted. The linear model can be easily extrapolated to include more toxicants by simply adding more terms obtained from different experiments; the nonlinear model is not as easily extrapolated, since the values of k and thus the independent variable described by the linear combination might differ between experiments. This difference is extremely important if some generalized method of predicting mixture toxicity based on additive components, such as the toxic units concept, is to be applied (more details later).

The experimental methodology used here was appropriate for testing hypotheses about the effects of mixtures with toxicants at different concentrations and ratios. In most experiments described in the literature, dilutions of only one mixture, with concentrations of toxicants proportional to their LC50's, were tested. Still, the hypotheses assumed to be confirmed were about the effects of toxicants at all possible ratios. In my experiments, only second-order interactions are examined and higher order interactions are confounded. In the usual design, all effects are described as one nth order interaction.

As mentioned previously, the toxicants in these experiments were chosen so that different types of physiological effects would occur, and hopefully, that different types of joint effects would be demonstrated. All three metal cations (Cu^{2+}, Cd^{2+}, and Zn^{2+}) are believed to act similarly at acutely lethal concentrations in that they cause precipitation and damage at the gill surface, ultimately leading to ionoregulatory problems. Cu^{2+} causes few structural changes but still impairs ionoregulation. Zn^{2+} does destroy gill architecture eventually leading to suffocation. Cd^{2+} additionally causes neurological dysfunction. Fish do acclimate to Cd^{2+}, and may acclimate better in the presence of sublethal levels of other metals due to enhanced metallothionein levels (Klaverkamp and Duncan 1987). The low concentrations of K^+ used in these experiments would upset the sodium–potassium balance in the nervous system. It is believed that the divalent ion Mg^{2+} competes with metals at active sites at cell surfaces and lessens toxicity. However, it is toxic in the hundreds of milligrams per litre range (Clarke 1974). LC50 concentrations for these five ions from various studies are given in Clarke (1974) and International Joint Commission (1981).

However, knowledge of physiological modes of action helps little in the interpretation of the results of experiments. There are no logical reasons why the effects of two toxicants acting differently or at different sites should not be described by equations for simple similar action or by equations with additive terms. Nor are there reasons why similar simple action should apply to any two of the above toxicants, since their physiological effects are similar but different. Also, mortality is an integrated and complex response; it does not seem reasonable that two toxicants would differ only in their strength of action. Moreover, although a certain combination of independent variables might describe one form of the response, a different description might be required for different transformations or descriptions of the response. Reporting joint lethal effects of

two compounds is as new as describing the effects of a new unknown compound. Very little information is available to place the data in these experiments into perspective.

The classifications of Ashford and Smith (1964) are perhaps the only ones that are general enough to relate expected physiological modes of joint action to the response surfaces that were obtained. These classifications do not describe how a chosen response variable is described by a combination of terms of the independent variables; they describe the instantaneous rate of change of independent variables in relation to each other in the chosen description of the response. Ashford and Smith (1964) believed that there are only two main classifications of the effects of toxicants: interactive and noninteractive. Noninteractive action is demonstrated for two toxicants if the criterion $(d^2R/dC_i dC_j)/((dR/dC_i)\cdot(dR/dC_j)) = f(R)$ (a function of the whole response surface equation) applies, or in other words, if the response surface can be expressed as modified response variable described by additive functions of each toxicant, namely as $f1(Y) = f2(C_i) + f3(C_j)$, in which $f1$, $f2$, and $f3$ are any functions. In the given response surfaces, noninteractive effects are described for all possible pairs of toxicants except for Cu and Cd in Equation (4). Equation (4) suggests a strongly enhanced toxic effect in the presence of both of these metals.

Simple similar action for two toxicants, a special case of noninteractive effects, can be demonstrated to occur if the response surface can be factored into the form $Y = f(a\cdot C_i + b\cdot C_j)$ or if $dC_i/dC_j =$ a constant. Several possible cases of simple similar action are described by the given response surfaces. For example, in both Equations (4) and (5), simple similar action applies to Cu and Zn if Cd is held constant. Simple similar action applies to K and Zn and Cu and K, if other ions are held constant, in all three described response surfaces. Examination of the response surfaces show that simple similar action is described many times, often only at constant levels of other toxicants.

Simple similar action is the mode of joint action that has been examined and confirmed most often. It has usually been tested with toxicants expected to act similarly, based on knowledge of their modes of action and on the fact that individual dose–response curves were parallel. Eighteen such cases are listed in de March (1987b), the most well known among these being Lloyd and Jordan (1963, 1964), Herbert and Shurben (1964), Sprague (1964), Brown (1968), Anderson and Weber (1975), Wong et al. (1978), Koenemann (1981), and Hermens and Leenwaugh (1982). The model has been rejected only once by Anderson and Weber (1975) for mixtures of Cu and Zn (but confirmed for Cu and Ni). All reported experiments were done with mixtures of equitoxic toxicant concentrations, and all results were assumed to confirm simple similar action in general. There were other experimental inadequacies: in all cases, fewer than eight mixture levels were tested, and only 4–10 test organisms were used at every experimental value. Thus, fit to expected modes of action was biased by small sample sizes.

The only method of rejecting the hypothesis of simple similar action applying to two toxicants would be to demonstrate that the model with a forced simple linear combination did not describe experimental results adequately. In my case, I would have to show that the forced model probit(p) = $(a\cdot\text{Cu} + b\cdot\text{Cd} + c)^2 + k$ did not describe experimental results adequately. In fact, in the Cu–Cd experiment examined alone, the hypothesis of simple similar action tested by fitting the above equation could not be rejected (model ($R^2 = 0.81$, all coefficients significant). However, the simple linear model with linear and quadratic terms as in Equation (5) had a significantly better fit ($R^2 = 0.90$) (generalized likelihood ratio test, Graybill 1976).

Another appealing mode of joint action not easily distinguished by the linear model is that of independent joint action with completely correlated susceptibilities. This concept, here used in a more generalized sense than defined by Bliss (1934), describes the case in which the effects of a toxicant are dependent on the presence, but not the concentration, of another. This concept might have been appropriate for describing the 48-h response (Equation (6)). Specifically, terms such as $a\cdot C_1\cdot r$, in which r is the correlation in susceptibilities applicable only in the presence of the toxicant 2, might have described the results as well as the cross-product, $b\cdot C_1\cdot C_2$.

Quantifying the degree of additivity, similarity, or interactivity of the effects of two toxicants with indices derived from response surfaces is a possibility arising from these experiments. I have shown (de March 1987a) that any model with a linear combination of terms can be expressed as a tolerance model. The simplest of these, the toxic units model, has appealed to toxicologists since it can be used to predict the levels of toxicants that will jointly produce the tolerance response (Brown 1968; Sprague 1970; International Joint Commission 1981). In a tolerance model a fixed level of a chosen form of the response variable, the tolerance response, for example probit(0.50), is partitioned by linear terms containing critial concentration values such as LC50's, threshhold concentrations, and mixture toxicity indices (as opposed to regression parameters such as slopes and intercepts used in the original linear model). When extrapolating a tolerance model to untested combinations, some of the critical concentration values required are available in the literature, while others can be obtained from experiments with subsets of the toxicants of interest, or be assumed from tests with similar toxicants.

A tolerance model derived from the response surface of the form

$$(7)\quad \text{Probit}(p) = \sum_{i=1,n} a_i\cdot C_i + \sum_{\substack{i=1,n\ j=1,n \\ i\neq j}} b_{ij}\cdot C_i\cdot C_j + k$$

would be

$$1 = \sum_{i=1,n} \frac{M_i}{O_i} + \sum_{\substack{i=1,n\ j=1,n \\ i\neq j}} \left[\text{MTI}_{ij}\cdot \frac{M_i\cdot M_j}{O_i\cdot O_j} \right]$$

in which M_1 to M_n are the concentrations of toxicants 1 to n that will jointly produce the tolerance response p'. In the linear model (Equations (4) to (6)) the form of the response that is partitioned would be (probit(0.50) $- k$) $= R'$. O_1 to O_n are "objective concentrations," the concentrations of toxicants that when used individually produce the tolerance response. MTI is a mixture toxicity index suggested in de March (1987a). It is one of several that can be calculated from regression coefficients and/or LC50's. The MTI required in the above tolerance model would be calculated as $\text{MTI}_{ij} = (R'\cdot b_{ij})/(a_i\cdot a_j)$, the coefficients obtained from the response surface of the same form as Equation (7). The MTI is zero if the effects are simply additive and is larger when the effects are more-than-additive. When the MTI is -1 and one of the M_i/O_i values is 1, then a special case of less-than-additive effects, specifically independent effects, in which only one toxicant appears to be effective is described (de March 1987a).

The 96-h MTI's, based on Equation (5), and assuming 10% control mortality (probit(0.50) − probit(0.10) = 5 − 3.7183 = 1.2817), would be 21.0 = (1.2817·331.1)/(2.552·7.910) for Cu and Cd and 10.8 = (1.2817·55.17)/(7.910·0.8289) for Cd and Zn. The MTI's would be larger if a smaller control mortality is assumed.

Other mixture toxicity indices have been described, for example those of Colby (1967), Marking and Dawson (1975), Burrell and Corke (1980), and Koenemann (1981). These indices are defined as a combination of LC50's of individual toxicants and component concentrations at the interpolated 50% response from experiments with mixtures of equitoxic concentrations. Again, the indices are then assumed to be indicative of all possible combinations of concentrations which cause 50% response even though they are obtained from dilutions of one mixture.

Koenemann (1981) defined a mixture toxicity index which has the value 1 for simple similar action and zero for independent action. My suggested index takes on the values zero and −1 for the same two modes of action, respectively. The two indices can be roughly compared if mine is scaled by adding 1 and then taking the logarithm to the base 10. In this case my two mixture toxicity indices are 1.34 for Cu and Cd and 1.07 for Cd and Zn, classifying the joint effects as slightly ("supraadditive" in Koenemann's classification system.

Marking and Dawson (1975) suggested a mixture toxicity index which is zero for additive toxicity and less than or greater than zero for less-than-additive or greater-than-additive toxicity. Rearrangement of his definitions of his index to one possible response surface, using methods similar to those in de March (1987a), shows that their index for more-than-additive toxicity is identical to my proposed index. Their index for less-than-additive toxicity is identical to an index calculated as $(c \cdot R')/(a \cdot b - c \cdot R')$ from Equation (7).

In general, my work has shown that resolving problems in the logic of describing joint effects of chemicals in mixtures may be more important than generating new data using dubious established methods. Practices such as the use of mixtures of toxicants at equitoxic concentrations to make generalizations about untested concentration ratios can easily be argued to be scientifically invalid. Similarly, the practice of defining mixture toxicity indices to summarize the results of a particular type of confounded experiment can be questioned. Confusing the many available definitions for "joint" or "interactive" effects, but derived from different models, from different possibilities within models, from relationships between either independent or dependent variables, or between specific or undefined response variables, seems to be characteristic of the field. Even if my experiments had smaller experimental errors, the choice of a multivariate model to describe data still would have been a serious decision which would not have been highly dependent on experimental results. The description of the degree of "interactivity" or "nonadditivity," or classifications such as simple similar action, would still be model dependent and for that reason alone would not necessarily concur with other work that had been done.

Even if a consistent system of models, experimental designs, and definitions of summary statistics appropriate for mixture effects is eventually accepted, the methods of application of such information to real contamination problems will still be of interest. For example, it will be interesting to observe whether the problems in bodies of waters polluted from many sources, such as the Great Lakes, will eventually be solved by referring to quantitative models for the effects of mixtures, and by what methods these models will acquire scientific defensibility.

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Effect of pH on Speciation and Toxicity of Aluminum to Rainbow Trout (*Salmo gairdneri*)

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Alum (aluminum sulphate) is used in many water treatment plants as a flocculant. The resultant settled floc (called alum sludge) may be discharged into receiving waters. This study reports the effect of pH on the speciation of released aluminum (Al) from fresh sludge and Al uptake and toxicity to fish in river water in the presence of abiotic substrates enclosed in dialysis sacs. This study shows that freshly precipitated alum sludge holds Al effectively within a wide range of pH (4.50–10.0) and that abiotic substrates compete with the biota as represented by fish for Al. Fish mortalities observed could be due to colloidal particles and acid- and Al-induced stress at pH 4.50, to colloidal particles at pH 6.00, and to high alkalinity at pH 10.0. At pH 7–9, Al in water, present essentially as filterable nonexchangeable Al (FNEX-Al), was not lethal to fish.

L'alun (sulfate d'aluminium) est utilisé comme agent de floculation dans bon nombre d'usines de traitement des eaux. Il arrive que le floc sédimenté ainsi obtenu (appelé boues à l'alun) soit rejeté dans des plans d'eau. L'auteur traite des effets du pH sur la spéciation de l'Al rejeté avec les boues, de même que de l'assimilation et de la toxicité de l'Al pour les poissons des cours d'eau en présence de substrats abiotiques en sacs de dialyse. L'étude montre que les boues à l'alun récemment précipitées conservent l'Al de façon efficace dans une large gamme de pH (4,50 – 10,0) et que les substrats abiotiques font concurrence au biote, représenté par les poissons, pour l'assimilation de l'Al. Les cas de mortalité observés chez les poissons peuvent s'expliquer par la présence de particules colloïdales, d'un stress induit par l'acidité et l'Al au pH 4,50, aux particules colloïdales au pH 6,00 et à la forte alcalinité au pH 10,0. Dans la gamme des pH 7 à 9, l'Al était essentiellement présent dans l'eau sous la forme d'aluminium filtrable non échangeable n'ayant pas d'effet létal pour les poissons.

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Elevated levels of aluminum (Al) may have serious effects on aquatic organisms, particularly fish under acidic stress. Dissolved Al, mobilized from sediments and suspended matter at increased acidities, was implicated in fish deaths in waters of pH 4.5–6.0 (Burrows 1977; Schofield and Trojnar 1980; Hutchinson and Sprague 1986). Surges of labile Al during snowmelt and heavy rainfall were shown to be potentially lethal to fish eggs and fry (Driscoll et al. 1980). It is known that complexation modifies the distribution and toxicity of trace metals (Pagenkopf et al. 1974; Giesy et al. 1977). Organic sequestration with citrate and humic matter was reported to reduce Al toxicity (Driscoll et al. 1980). Aluminum sludge, formed during water treatment plant processes, contains high concentration of Al and is periodically discharged into surface waters. Although exchange of Al and other metals between water–sediment sytems has been studied, very little is known about Al exchanges in multisubstrate systems which are environmentally more relevant. Hence, a study on the effect of pH on the release of Al from the sludge, its speciation in river water, and toxicity to fish in the presence of environmentally relevant abiotic substrates would enable us to evaluate the impact of alum sludge on receiving waters.

Since in the natural aquatic environment, metals including Al are mostly partitioned between sediment and water, it was decided to follow the Al flux from alum sludge as a function of pH into the overlying water, and to assess its subsequent toxicity to rainbow trout under conditions similar to natural and polluted waters. Hence in this study an elaborate multisubstrate experimental system was used to provide abiotic interactions from surface-active solid phases to compete with biota as represented by rainbow trout (*Salmo gairdneri*) in the partitioning of Al mobilized from alum sludge. In addition, Al in water was quantitatively speciated as a function of pH and the toxic and nontoxic Al species were identified from fish mortality data.

Materials and Methods

Experimental Design and Apparatus

A laboratory simulation of an aquatic ecosystem was used in this study (Fig. 1). Fresh alum sludge from a water treatment plant on the North Saskatchewan River (NSR) in Edmonton, Alberta, Canada, was the source of Al. The Al content of the alum sludge was analyzed to be 108 000 mg/kg. Abiotic

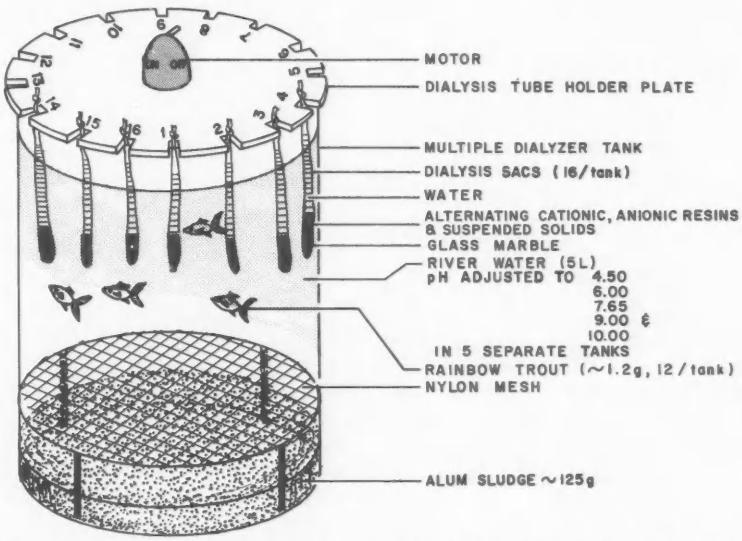
MOTOR
DIALYSIS TUBE HOLDER PLATE
MULTIPLE DIALYZER TANK
DIALYSIS SACS (16/tank)
WATER
ALTERNATING CATIONIC, ANIONIC RESINS & SUSPENDED SOLIDS
GLASS MARBLE
RIVER WATER (5L) pH ADJUSTED TO 4.50 6.00 7.65 9.00 & 10.00 IN 5 SEPARATE TANKS
RAINBOW TROUT (~1.2g, 12/tank)
NYLON MESH
ALUM SLUDGE ~125g

FIG. 1. Multisubstrate exposure system for the study of Al speciation in river water and its effect on fish in presence of competing compartments.

substrates chosen to compete for Al with the biotic substrate (rainbow trout) were (1) filtered NSR water, (2) suspended solids (SS), (3) cation-exchange resin (CER), and (4) anion-exchange resin (AER). The ion-exchange resins were chosen as substrates to represent the naturally occurring ion-exchangeable polyelectrolyte materials in the aquatic environment. These substrates were placed as separate entities in a 5.5-L borosilicate glass tank (exposure tank) and the river water acted as the fluid transfer medium for Al between substrates.

Characteristics of the Substrates

Water

Five litres of NSR water was contained and continuously stirred in the exposure tank (Fig. 1). Relevant characteristics of the filtered NSR water at a typical pH of 7.65 are as follows: conductivity = 375 μS, total hardness = 149, K = 1.1, F = 0.12, SO_4 = 38, total alkalinity = 119, NH_3 = 0.004, total Kjeldahl nitrogen = 0.22, total dissolved solids = 174, Na = 5, Cl < 1.0, bicarbonate = 143, Ca = 40, dissolved organic carbon = 4.2, dissolved inorganic carbon = 37.0, phosphate ≤ 0.002, and $NO_2 + NO_3$ = 0.003 (all concentrations in milligrams per litre). Trace metal concentrations (milligrams per litre) were as follows: Hg < 0.001, Cu < 0.001, Cd < 0.001, Zn = 0.010, Co < 0.001, Pb = 0.005, Mn < 0.008, Ni = 0.010, and Al = 0.040.

Suspended solids (SS)

Pulverized montmorillonite clay was used as SS and 1 g of pulverized clay was placed with 5 mL of deionized water in each dialysis sac. There were six dialysis sacs containing SS in each exposure tank (Fig. 1).

Cation-exchange resins (CER)

Amberlite IR-120 (H) resin was used as the CER in this study. The resin was swelled in deionized water prior to use and 2 g of CER with 5 mL of deionized water was placed in each dialysis sac. Thus, there were six dialysis sacs containing CER in each exposure tank.

Anion-exchange resins (AER)

Amberlite IR-45 (OH) resin was used as the AER in this study. The resin was swelled in deionized water prior to use and 1 g of AER with 5 mL of deionized water was placed in each dialysis sac. Once again, there were six dialysis sacs containing AER in each exposure tank (Fig. 1).

Dialysis sacs

The dialysis sacs were chosen to contain the abiotic substrates SS, CER, and AER physically separate from each other. This prevented the mixing of abiotic substrates in the exposure tank. The average pore diameter of the dialysis sac membrane was 4.8 nm and the membrane was permeable to metals and their complexes of molecular weight below 12 000 (Ramamoorthy and Blumhagen 1984). There were a total of 18 dialysis sacs in each exposure tank: 6 sacs containing SS, 6 sacs containing CER, and 6 sacs containing AER.

Fish

Rainbow trout hatched from eggs in our laboratory were used in the study. Twelve fish with an average weight of 1.2 g (SD ± 0.2 g) and about 20 wk old were placed in each exposure tank. Fish were not fed during the experiment.

Exposure Designs and Sampling

Exposures

(1) The alum sludge (125 g) was kept at the bottom of the exposure tank by covering it with a nylon mesh (Fig. 1). The abiotic substrates enclosed in dialysis sacs were suspended in the continuously stirred NSR water. Thus the abiotic substrates and fish were exposed to Al, mobilized from the alum sludge. Five exposure tanks were set up identically with respect to alum sludge and abiotic and biotic substrates. The only variation among the five exposure tanks was the pH chosen. The pH levels chosen were 4.50, 6.00, 7.65 (natural pH of NSR water,

not adjusted), 9.00, and 10.0. The pH was adjusted manually by adding concentrated HCl or saturated NaOH solution using a micropipette.

(2) An additional five exposure tanks were set at pH 4.50, 6.00, 7.65, 9.00, and 10.0 with conditions similar to design 1 but without alum sludge. These exposures served as controls and were set up to determine the effect of pH alone on fish in the absence of Al. Thus, by comparing the results of these two sets of exposures, the effect of pH alone and effect of Al + pH on fish could be determined.

(3) A third set of exposure tanks was set up with conditions similar to design 1. The only difference was to enclose the alum sludge in dialysis sacs and to place them at the bottom of each exposure tank. This exposure was designed to determine the toxic role of suspended colloidal particles originating from the alum sludge. The pore size of dialysis sacs (4.8 nm) prevented the colloidal particles from escaping the alum sludge and entering the water in the exposure tank. Thus, comparison of results from exposure design 3 with exposure design 1 permits the assessment of the toxic role of colloidal particles originating from the sludge.

Sampling

At sampling times of 3, 6, 12, 24, 48, and 96 h, one sac each of SS, CER, AER, two fish, and two water samples (100 mL each) were removed from each tank for Al analysis. One water sample was analysed for total Al and the other for Al speciation (Fig. 2).

Experimental Speciation of Al in NSR Water from Exposure Tanks

(1) A water sample (100 mL) from each exposure tank was collected and centrifuged with a table centrifuge to remove the particulate matter, if any. The supernatent NSR water was taken for further treatment and the separated particulate matter for Al analysis. The particulate matter was termed residue (I) (Fig. 2, Step 1). Centrifugation was used instead of filtration to avoid possible sorption of Al to filter papers and release of anions such as phosphate and acetate from filter papers which could alter the speciation of Al. It has been recently reported by Driscoll (1984) that filtration might fail to remove suspended microcrystals of aluminum hydroxide. The technique of complexation of 8-hydroxyquinoline with rapid solvent extraction, as used in this study, is selective to *dissolved monomeric Al* in water (Driscoll 1984, and references cited therein).

(2) The supernatant NSR water from Step 1 (free from particulate matter) was eluted through the CER column and collected in a conical flask (Fig. 2, Step 2). This eluate was free from filterable cation-exchangeable Al (FCEX-Al). The pH drop during elution depended on [FCEX-Al] exchanging for H^+ from the resin. At pH 4.5, most of the FCEX-Al was on the particulate matter and the *dissolved* [FCEX-Al] was too low to

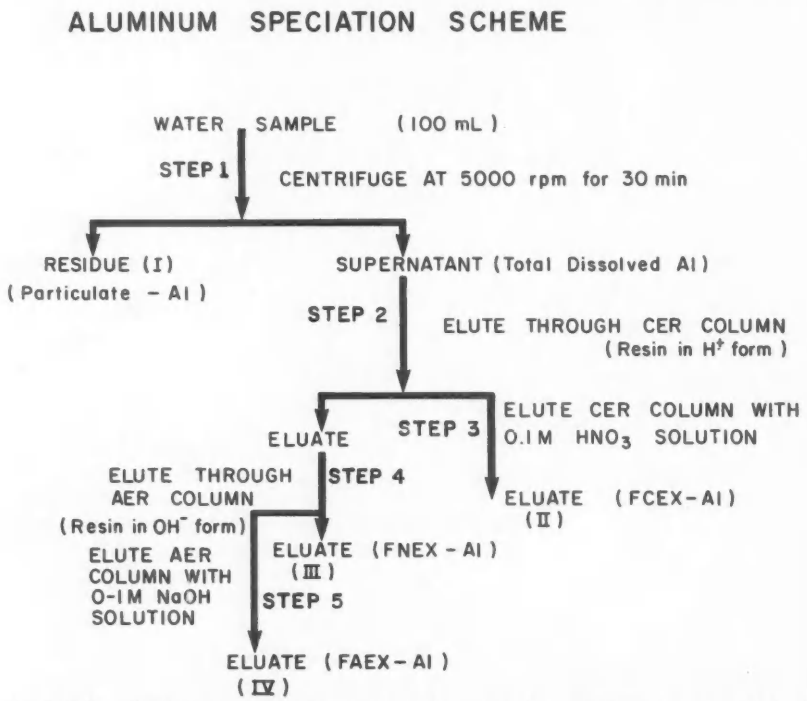

FIG. 2. Schematic diagram showing the experimental sequence for separation of species of Al. pH values can be deduced from the concentrations of the eluants mentioned. See text for the type of counter ion for CER and AER used in this study. Mass balancing of total Al (particulate + dissolved) with the sum of Al from all fractions was carried out. The percent recovery was 96–103%. FAEX-Al fraction gave occasional unsatisfactory recoveries, and in such cases, the calculated FAEX-Al ([$= T_{Al}$ minus (particulate − Al + FCEX − Al + FNEX − Al)] value was used, since the recovery was consistent for all other fractions.

cause any substantial change in pH during elution. At pH 7.65 and 9.0, the total [FCEX-Al] was 2.24 and 24.6 μg in 100 mL of sample, respectively. The pH of the eluate was restored to its initial value by progressive addition of aqueous sodium hydroxide (0.1 M).

(3) The CER column was eluted with 0.1 M nitric acid solution three times with 20 mL each time, followed by two rinsings with 20 mL of nanopure water (NPW) and collected in a polypropylene (PP) bottle (Fig. 2, Step 3). This was the FCEX-Al (II in Fig. 2).

(4) The eluate from Step 2 was eluted through the AER column and collected with two NPW rinses in a PP bottle (Fig. 2, Step 4). The eluate from this Step 4 contained the filterable nonexchangeable Al (FNEX-Al) (III in Fig. 2), free from both cationic and anionic Al species. The pH increase corresponded to [filterable anion exchangeable Al (FAEX-Al)] exchanging for OH^- from the resin. The total FAEX-Al (micrograms) at different pH was as follows: 113 (pH 4.50), 6.4 (pH 7.65), 86 (pH 9.00), and 1472 (pH 10). Excepting pH 10, the pH change was not excessive because of dilution from rinses. pH was restored to its initial value by progressive addition of aqueous nitric acid (0.1 M). Volume was recorded for every sample for accurate mass-balancing of Al.

(5) The AER column was eluted with 0.1 M NaOH solution and collected with rinsings of NPW in a PP bottle (Fig. 2, Step 5). This was the FAEX-Al fraction (IV in Fig. 2).

(6) Thus, there were four Al analyses in each speciation train: one residue and three eluates (FCEX-Al, FAEX-Al, and FNEX-Al).

Analytical readings were corrected for background Al levels in AER, SS, and NSR water to arrive at the net Al concentration. It should be noted that the matrix-bound Al in AER and SS (which is an integral part of their crystal structure) was not leachable under the experimental conditions. Only under drastic analytical conditions was Al released from the matrices of AER and SS.

Depending on the type of matrix, Al was analyzed by atomic absorption spectrophotometry (AAS) using direct aspiration, a graphite tube furnace (U.S. Environmental Protection Agency 1979), or a solvent extraction method using methyl isobutyl ketone (MIBK) (Anonymous 1986).

Aluminum Analysis in Source Material and Substrates

Alum sludge

Alum sludge, the source of Al in the study, was analyzed by the following method. Half a gram of the alum sludge was weighed into a Teflon beaker; a mixture of 10 mL HF + 3 mL $HClO_4$ + 3 mL HNO_3 (all concentrated) was added and the mixture heated to dryness. Two millilitres of $HClO_4$ was added and the mixture heated to dryness again. The residue was dissolved in 3 mL of HNO_3, 10–15 mL of deionized water was added, and this was heated on a hot plate for 5–10 min for complete dissolution. The solution was cooled, made up to a known volume and aspirated into the nitrous oxide – acetylene flame of an AAS for Al determination (U.S. Environmental Protection Agency 1979).

Water

Water samples were analyzed by solvent extraction method (Anonymous 1986) using MIBK and 8-hydroxyquinoline. The water sample was extracted after chelation with 8-hydroxyguinoline into MIBK. The MIBK extract was then aspirated directly into a nitrous oxide – acetylene flame of an AAS for Al determination.

Suspended solids

Suspended solid samples were analyzed by the same method as described for alum sludge.

Resins

Half a gram of resin sample was weighed into a beaker, 5 mL of concentrated HNO_3 added, and this was heated to about 0.5 mL of residual volume. One millilitre of HNO_3 and 15 mL of water were added and heated on a hot plate for 5–10 min. The solution was cooled, made up to a known volume, and analyzed for Al. Cationic resin sample was analyzed by graphite tube furnace (Varian GTA 95) on a Varian 475 AAS. The samples were charred at 1400°C for 3 s and atomized at 2500°C in the graphite tube furnace. Anionic resin samples were analyzed by the solvent extraction method (U.S. Environmental Protection Agency 1979; Anonymous 1986) using a Varian 475 AAS.

Fish

Whole fish was weighed into a beaker, 10 mL of concentrated HNO_3 was added, and the contents heated gently until the fish decomposed. Heating was continued until the sample was dry and then ashed at 400°C in a muffle furnace to remove organic matter. The residue was cooled, dissolved in 2 mL of concentrated HNO_3, made up to a known volume, and analyzed by the solvent extraction method (Anonymous 1986) using an AAS.

Detection limits and recovery

Method	Detection limit	Recovery
Solvent extraction method	0.005 μg/g or μg/mL	95%
Graphite tube furnace method	0.001 μg/g or μg/mL adjusted and matrix matched	95% with background

A Scan for metals other than aluminum showed nondetectable amounts in substrates. Every batch of samples analyzed for Al included 15–20% of quality control samples and synthetic standards for quality assurance of analytical data. All Al analyses were carried out by Chemex Laboratories (Calgary, Alberta).

Mass Balancing of Al in Exposure Tanks

Substrates

To calculate the total amount of Al released from the alum sludge, a mass balance of Al content in all substrates was carried out. This was done by multiplying the concentration of Al in each substrate by the mass of that substrate. For example, the total mass of Al retained in water at pH 4.50 was calculated by multiplying the concentration of 5.65 μg/mL by its total mass of 5 kg (5 L). Similarly the mass content of Al in each substrate (CER, AER, and fish) was calculated by multiplying the concentration of Al (micrograms per gram) by the total mass of each substrate. The sum of Al content in all substrates (both abiotic and biotic) yielded the total quantity of Al released from the sludge.

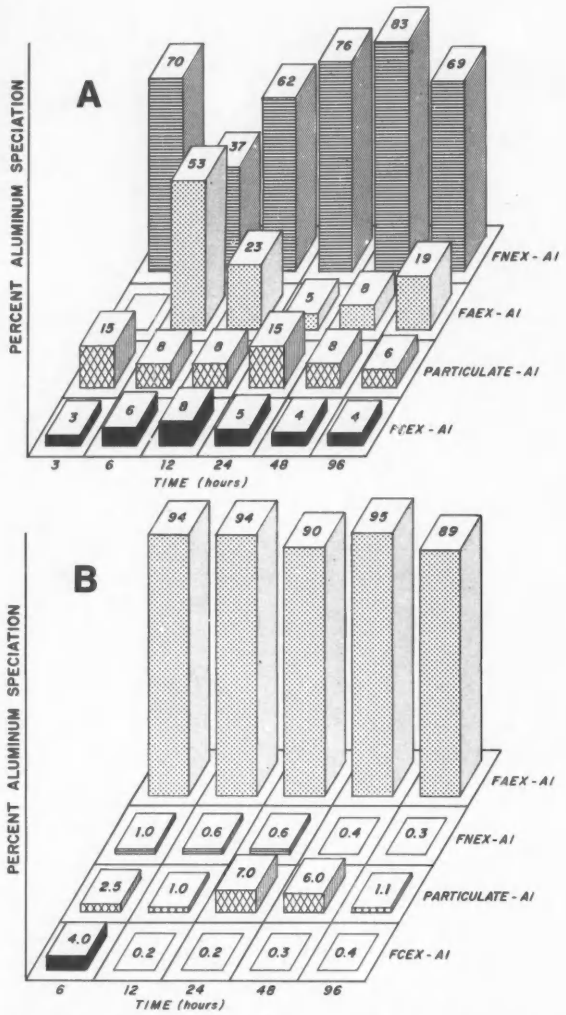

FIG. 3. Three-dimensional plot of the concentrations of Al species in water as a function of sampling times (3, 6, 12, 24, 48, and 96 h) for (A) pH = 7.65 and (B) pH = 10.0 (units = percentage concentrations). ■, filterable cation-exchangeable Al species (FCEX-Al); ▨, particulate matter bound Al species (particulate Al); ▒, filterable anion-exchangeable Al species (FAEX-Al); ▤, filterable nonexchangeable Al species (FNEX-Al).

Speciation

There were two water samples taken at each sampling time. One water sample without any speciation treatment was analyzed for total Al. The other water sample was speciated according to the scheme given in Fig. 2. All the species identified in water through the speciation scheme were individually quantified on a mass basis by multiplying its concentration with its total volume. The sum of all filterable species was equal to the total dissolved Al. Finally, for a given sampling time, the total Al from nonspeciated water sample was balanced with the total Al from speciated water sample to calculate the recovery of Al for the speciation scheme. The percent recovery was 96–103%.

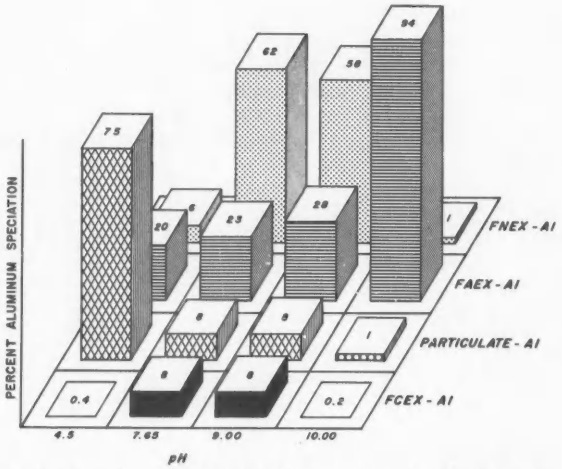

FIG. 4. Three-dimensional plot of Al species in water and their concentrations as a function of pH at 12 h (units = percentage concentrations). ■, filterable cation-exchangeable Al species (FCEX-Al); ▨, particulate matter bound Al species (particulate Al); ▤, filterable anion-exchangeable Al species (FAEX-Al); ▒, filterable nonexchangeable Al species (FNEX-Al).

Speciation procedures for metals with mass balancing of the metal distribution among various species have been reported (Ramamoorthy and Kushner 1975; Hart and Davies 1981; Campbell et al. 1984). A review of heavy metal speciation in natural waters is also available (Morrison 1983).

Results

Changes in Al Concentration with Exposure Time

The abiotic and biotic substrates sampled from each exposure tank at 3, 6, 12, 24, 48, and 96 h were analyzed for Al content, following appropriate digestion and analytical procedures as described above. At each sampling time, the Al content in each substrate represented the extent of Al uptake. The sum of uptake by all substrates corresponded to the amount of Al mobilized from the alum sludge as a function of exposure time. The analytical results showed that the speciation of Al in water did not change from 12 h to the end of the experiment (96 h) (Fig. 3). Hence, results for the experimental speciation of Al in water as a function of pH from 4.50 to 10.0 are given for a typical sampling time of 12 h (Fig. 4).

The uptake of Al by fish seems to reach a peak in 12 h. However, average values were given for the uptake of Al by fish, since fish seems to clear some of the accumulated Al (Table 1). Similarly, results given for the uptake of Al by abiotic substrates are average values (Table 2).

The pH was measured at every sampling time, prior to adjustment, in all exposure tanks. The maximum variation in pH between adjustments at sampling times was −0.81 at pH 9.00 (Table 1).

Al Speciation in Water

At all pH values and sampling times, the concentration of Al on a relative scale was the lowest in water of all substrates. However, on an absolute mass scale, the Al content was highest in water (Table 2).

TABLE 1. Aluminum uptake by fish exposed to alum sludge at different pHs and time intervals. *M* = mortality of fish; — = all fish died prior to sampling time.

	pH[a] (range[b])						
	4.50 (+0.55)	6.00 (+0.45)	7.65 (±0.10)		9.00 (−0.81)		10.0 (−0.25)
Time (h)	Al uptake (μg/g)[c]	Al uptake (μg/g)[c]	Initial biomass (g. wet)	Al uptake (μg/g)[d]	Initial biomass (g. wet)	Al uptake (μg/g)[d]	Al uptake (μg/g)[c]
3	*M* = 12	32.51 *M* = 4	2.03	4.26	2.84	5.29	22.61
6	—	*M* = 8	2.82	4.72	1.80	5.60	*M* = 7
12	—	—	1.40	6.86	1.64	4.14	*M* = 5
24	—	—	2.10	2.93	1.40	4.07	—
48	—	—	1.60	2.56	2.30	5.04	—
96	—	—	3.10	2.34	1.70	12.04	—
Average uptake[c,d]	—	32.5		3.9		6.0	22.6

[a]Measured pH.

[b]Variation in pH between adjustments at sampling times (3,6,12, and 24 h) and after, at 12-h intervals. Variations tended to be smaller after 24 h of sampling time (e.g. −0.40 and −0.10 for pH 9.00 and 10.0, respectively).

[c]In pH regimes causing fish mortality, the sampling biomass might include resident water due to rapid influx when they are either moribund or dead. Hence, uptake data at pH 4.5, 6.0, and 10.0 should be treated with caution.

[d]At pH 7.65 and 9.00, fish were alert during the entire exposure period. The uptake data (μg Al/g biomass (wet)) was calculated using the initial fish biomass and correcting for any water uptake to arrive at the true tissue Al concentration.

TABLE 2. Average Al uptake by fish and competing substrates exposed to alum sludge at varying pHs. — = true biomass could not be calculated, since fish were moribund or dead.

	Concentration of Al in				Total Al (mg) on mass balance basis	% Al retained in water
Exposure pH	Fish (μg/mL)	Cation resin (μg/g)	Anion resin (μg/g)[b]	Water (μg/mL)		
4.50	—	71.40 (3.5)[a]	20.0 (12.6)[a]	5.65	29.23	96.6
6.00[c]	32.5	3.40 (1.6)[a]	70.0 (0.1)[a]	44.28	222.04	99.7
7.65	3.9	1.54 (71.0)[a]	20.0 (5.5)[a]	0.28	1.59	88.0
9.00	6.0	6.54 (19.5)[a]	60.0 (2.1)[a]	3.07	15.86	96.8
10.0	22.6	19.28 (12.8)[a]	200.0 (1.2)[a]	15.66	79.77	98.2

[a]Partition coefficient between compartment and water

[b]Corrected for background Al level

[c]Turbidity at all sampling times

Circumneutral pH (6.00 and 7.65)

At pH 6.00, 222 mg of Al was released from the sludge and 99.7% of it resided in water. Particulate Al in water was the predominant species at pH 6.00 and accounted for 44.28 μg/mL of the total Al (Table 2). At pH 7.65, total Al released from the sludge was 1.59 mg, calculated from mass balancing all substrates. The individual weights of substrates were as follows: water = 5 kg (5000 mL), CER (2 g × 6 sacs) = 12 g, AER (1 g × 6 sacs) = 6 g, and fish = 12.18 g. The total Al was found to be distributed as follows: water = 88%, AER = 7.5%; CER = 1.2%, and fish = 0.03%.

The Al left in water at pH 7.65 (Fig. 4) was speciated as FNEX-Al (62–69%), FAEX-Al (23–19%), particulate Al (8–6%), and FCEX-Al (8–9%). The finding that FNEX-Al species (nonlabile Al) predominate at neutral pH is consistent with field data reported in the literature (Driscoll et al. 1980; Campbell et al. 1984).

Acidic pH (4.50)

Total mass balance of Al in all substrates taking into account their individual weights showed that 29.23 mg of Al was released from the sludge at pH 4.50. In pH regimes causing fish mortality, such as pH 4.5, the biomass sampled could be much higher than the initial biomass due to massive influx of

water containing Al. In other words, active uptake of Al was questionable at pH 4.5, since fish were not alert. In such cases, calculation of total Al in fish could be erroneous because the concentration of Al in fish extract might include the passively influxed Al via water. Therefore, ignoring the uptake by fish, the percentages of Al in CER and water were about 2.9 and 97% of the total Al mobilized from alum sludge (Table 2). The percent contribution by fish to total Al has already been shown to be small (0.03% in circumneutral pH of 7.65).

Speciation of Al retained by water at 12 h (Fig. 4) showed that at pH 4.5, particulate Al was dominant (74–75%), followed by FAEX-Al (24–20%), FNEX-Al (2–6%), and FCEX-Al (<1%). The "apparently low" concentration of FCEX-Al (Fig. 4) in water is due to its sorption to particulate matter and subsequent appearance as particulate Al fraction (74–75%).

Protonation of alum sludge particles leading to stable colloids is the likely cause for the relatively high concentration of particulate Al in water at pH 4.50 (Letterman and Iyer 1985). Either as simple or mixed chelates, alumino-organic complexes have a range of stabilities (Manning and Ramamoorthy 1972; Ramamoorthy and Manning 1974) and thus could pass through cationic resins in the speciation scheme. Driscoll (1984) observed this phenomenon with aluminum citrate which passed through cation exchange resins.

Alkaline pH (9.00 and 10.0)

Total mass balanced Al was 15.86 mg at pH 9 and 79.77 mg at pH 10 (Table 2). The percent distribution of Al among the substrates at pH 9.00 and 10.0 is as follows: water = 96.8%, CER = 0.49%, AER = 2.27%, and fish = 0.46% at pH 9.00; water = 98.2%, CER = 0.29%, and AER = 1.5% at pH 10.0. Once again, the Al uptake in fish at pH 10.0 could not be calculated accurately, since there was 100% mortality in 12 h of exposure (Table 1). At pH 9, some nonlabile organic Al, probably of higher stability, was also present, passing through the anionic exchange column and ending up as FNEX-Al (Fig. 4).

At pH 9.00, 15.38 mg (~97%) of the total Al left in the water column was speciated as FNEX-Al (58%), followed by FAEX-Al (28%) (Fig. 4). At pH 10.0, FAEX-Al was predominant (94%) with Al in water at 78.30 mg (Table 2; Fig. 4). Since dissolved organic carbon in NSR water is too low (4 mg/L) to account for all FAEX-Al at pH 10.0 (94% of 15.66 mg/L (or μg/mL), Table 2), it is likely that some organically chelated Al was released from the sludge along with free Al ions as $Al(OH)_4^-$ species.

Uptake of Al by Abiotic Substrates

Abiotic substrates competed effectively with fish for Al. Water retained the highest percentage of the total Al mobilized from alum sludge at all pH values (Table 2). Speciation of the Al retained in water showed that the dominant species at different pHs were as follows: (i) at pH 4.50, the particulate-bound FCEX-Al (74–75%), (ii) at pH 7.65 (natural pH of NSR water), FNEX-Al (62–69%) followed by FAEX-Al (23–19%), (iii) at pH 9.00, FNEX-Al (58%) followed by FAEX-Al (28%), and (iv) at pH 10.0, FAEX-Al (94%). Cation-exchange resin enclosed in dialysis sacs competed with fish for Al at pH 4.50 and AER at pH ≥ 7.65 (Table 2). Greater competition for Al by AER (6–10 times) in the pH region 7–9 minimized the amount of Al available for fish. Because montmorillonite clay (SS) contained a higher concentration of native Al than expected, it was difficult to quantify the sorbed Al above the background level. Hence, results for SS substrates were not included in Table 2.

Uptake of Al by Fish

The uptake of Al by fish at pH 7.65 seems to reach a peak (6.86 μg/g biomass) in 12 h and subsequently declines for sampling times at 24 h (2.93 μg/g), 48 h (2.56 μg/g), and 96 h (2.34 μg/g) (Table 1). For pH 9.00, the uptake of Al is more or less even (excepting the last sampling time of 96 h) at 4.83 ± 0.77 μg/g biomass (Table 1). Aluminum determined in fish was lowest (3.9 μg/g) at pH 7.65 and increased on either side of this pH. It should be noted that with time, fish seem to clear some of the accumulated Al (Table 1).

Effects of Al on Fish at Different pHs

Effect of Al and H^+

In the presence of alum sludge, fish mortalities occurred at pH 4.50, 6.00, and 10.0 (Table 1). Since most Al was particulate bound at pH 4.50 and 6.00, it is possible that fish died of suffocation due to finely dispersed Al colloids sorbing to the surface of gill epithelium (J. H. Youson and C. M. Neville, pers. comm.). In addition, both H^+- and Al-induced stress causing gill damage and ionic imbalance could have contributed to fish mortality at pH 4.50 (Schofield and Trojnar 1980; Neville 1985).

Effect of H^+ alone

In the "pH only" control, fish mortalities occurred at pH 4.50 and pH 10.0 only. At pH 4.50, there was only 8% mortality (one fish) after 24 h of exposure with no further mortalities at 96 h (Table 3). These results are consistent with the literature values (Neville 1985; McDonald 1983). In the "pH only" control at pH 10, 70–80% of the total number of fish died at 3 h of exposure and the rest at 6 h. This control was replicated three times and others twice. There were no fish mortalities at pH 6.0, 7.65, and 10.0 (Table 3).

Effect of colloidal Al particles

In separate experiments where the alum sludge was kept enclosed in dialysis sacs at the bottom of exposure tanks (other experimental conditions including pH regimes being similar), there were fish mortalities at pH 4.50 and pH 10.0 (Table 3). At pH 4.50, only 6% of the total number of fish died during 48 h and 20% died during 96 h. The remaining (74%) fish survived the exposure to Al at pH 4.50 whereas at pH 10.0, 67% of the total number of fish exposed (12) died at 3 h and the remaining fish (33%) died at 6 h of exposure (Table 3). Enclosing of sludge in dialysis sacs prevented the colloidal particles escaping from the sludge. There were no fish mortalities at pH 6.0, 7.65, and 10.0.

From the results, it can be concluded that fish mortalities observed could be due to colloidal particles and acid- and Al-induced stress at pH 4.50, to colloidal particles at pH 6.00, and to high alkalinity at pH 10.0 (Table 3). At pH 7–9, Al in water, present essentially as FNEX-Al, was not lethal to fish.

Discussion

Level of Dissolved Al in Water

The relatively higher levels of dissolved Al reported in this study are likely due to elevated levels of complexing anions present in the NSR water such as sulphate (38 mg/L), humic

TABLE 3. Mortality of fish at different exposures and pHs. Total number of fish in each exposure tank = 12; length of exposure period = 96 h; *M* = mortality; NM = no mortality; number in parentheses refers to the time of mortality.

Exposure design	pH				
	4.50	6.00	7.65	9.00	10.0
Design 1. Open alum sludge	*M* = 12 (3 h), All fish dead	*M* = 4 (3 h) All fish dead	NM	NM	*M* = 7 (6 h) *M* = 5 (12 h) All fish dead
Design 2. pH variation only without alum sludge present	*M* = 1 (24 h) Rest survived	NM	NM	NM	*M* = 9 (3 h) *M* = 3 (6 h) All fish dead
Design 3. Alum sludge enclosed in dialysis sacs	*M* = 1 (48 h) *M* = 2 (96 h) Rest survived	NM	NM	NM	*M* = 8 (3 h) *M* = 4 (6 h) All fish dead

and fulvic acids (dissolved organic matter), etc. In this study, the "dissolved Al" refers to all filterable species of Al which includes FCEX-Al, FNEX-Al, and FAEX-Al (Fig. 2). Al forms strong coordinate water-soluble complexes with organic and inorganic ligands (Pytkowicz 1983). Salts of Al with strong acids are fairly soluble, for example, $Al_2(SO_4)_3$ = 31.3 g/100 mL water (Weast and Astle 1982–83). Depending on the complexing capacity of the water, the amount of dissolved Al in equilibrium with solid aluminum hydroxide will increase. In two respects this study is different from most other field studies conducted in the northeastern United States and Canada on Al in natural waters. Firstly, unlike prairie waters, the surface waters of the Precambrian Shield are soft and low in metal-complexing capacity. Secondly, the source of Al is different. The latter studies measured the level of Al leached naturally from silicate minerals whereas the present study reports the resolubilization of Al from freshly flocculated synthetic alum sludge. The solubility of Al from freshly precipitated sludges is higher than the level expected from gibbsite (Al_2O_3), the reason being that Al exists as amorphous aluminum hydroxide in freshly precipitated sludge and is more reactive and more soluble than the crystallized gibbsite. The crystallization of aluminum hydroxide to gibbsite is slow and may take a year or more (Anonymous 1987), with solubility decreasing at a corresponding rate. Often, plots of Al in equilibrium with gibbsite are used in calculating dissolved Al levels, which is valid only for aged gibbsite as Al source. This topic has been discussed in detail in recent publications (Anonymous 1987; Letterman and Iyer 1985).

Analytical data showed that Al speciation changed with changes in pH. Aluminum shifted from predominantly particulate-bound Al species at acidic pH 4.50 to nonexchangeable Al species (FNEX-Al) in the pH range 7.65–9.00 and finally to anion-exchangeable Al species (FAEX-Al) at alkaline pH 10.0 (Fig. 4).

Level of Al in Abiotic Substrates

Abiotic substrates competed effectively with fish for Al. CER enclosed in dialysis sacs competed with fish for Al at pH 4.50 and AER at pH ≥ 7.65 (Table 2). Greater competition for Al by AER (6–10 times) in the pH region 7–9 minimized the amount of Al available for fish.

Effect of Al on Fish at Different pHs

The fish mortality data from the three different exposure designs (described earlier) provided some insight into the understanding of the modes of toxicity of alum sludge to fish in this study (Table 3).

(1) Fish mortalities observed at pH 4.50 could be due to colloidal particles and acid- and Al-induced stress.

(2) Finely dispersed Al colloids orginating from the alum sludge were acutely lethal to fish at 6.00. Accumulation of Al on the surface and in the cytoplasm of gills in rainbow trout has been shown by electron microscopy and X-ray diffraction studies (Youson and Neville 1987). Maximum toxicity at pH 6.5 was attributed to an insoluble form of Al in *Daphnia magna* (Havas 1985) and to a transient formation of fine particulate Al at pH 6.1 in rainbow trout (Neville 1985). In the latter there was no mortality at pH 6.5.

(3) Fish mortality at pH 10.0 may be due to a number of factors including caustic action on gills and ion imbalance with concomitant changes in osmoregulation. Base addition could have released the organic bound Al from sludge, accounting for the predominance of FAEX-Al species at pH 10.0. Comparison of mortality data for control fish with "pH + alum sludge" fish at pH 10.0 (Table 3) suggests that FAEX-Al might be offering some transient protection to fish in terms of increasing their longevity by a few hours.

(4) FNEX-Al, predominating at pH 7–9, is obviously nonlethal to fish. This is consistent with the earlier suggestion that organic sequestration of Al might eliminate its toxicity to fish and with the field observation of fish survival at moderately low pH in Adirondack lakes where nonlabile Al predominates (Driscoll et al. 1980).

Although losses of fish populations in acidic environments have been reported in Scandinavia and North America over a decade, implication of Al as a toxic agent was suggested only recently (Schofield and Trojnar 1980; Dickson 1978). However, speciation of Al was not considered in all these studies. A study on the characterization of Al in acidic lakes in granitic

ecosystems identified the inorganic labile Al species as the toxic species (Driscoll et al. 1980). Subsequent Al toxicity studies dealt with exposure of fish to synthetic inorganic Al without any Al speciation.

The present study is relevant to the natural aquatic environment and hence different from earlier studies on the following aspects: (i) the source of Al was an environmental sample and not a synthetic Al chemical, (ii) the pH range was wide (pH 4.5–10.0) and hence the results are applicable in interpreting Al chemistry to a range of aquatic environments, for example from fish kills in acidic lakes to natural waters at circumneutral pH and to surface waters receiving alum sludge discharges from wastewater treatment plants, (iii) detailed Al speciation in tandem with toxicity assessment of species in a wide pH range was carried out, and (iv) inclusion of abiotic substrates in the exposure of fish to Al simulated the natural aquatic environment where the uptake of Al is likely to be competitive.

We have a very limited knowledge of the physiology of gill interaction in Al uptake and toxicity, yet these organism–Al–pH interactions have great environmental relevance. Results of this study show that negligible amounts of Al are released from the alum sludge in the pH range of alum discharge into surface waters from wastewater treatment plants. If the turbidity is low, Al is not toxic even at lower pH's. This is evident from the fish mortality results of "pH only" exposure and also from exposure where turbidity from colloidal particles was eliminated (Table 3). The results show a 74% fish survival at pH 4.50 and 100% fish survival at pH 6.00, if the turbidity from colloidal Al particles is kept low. The results from this study could explain Al toxicity to fish in acidic lakes, especially during and after snowmelt and rainfall when turbidity may be high.

Acknowledgements

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Mitochondrial DNA Variation in Great Lakes Walleye (*Stizostedion vitreum*) Populations

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Billington, N., and P. D. N. Hebert. 1988. Mitochondrial DNA variation in Great Lakes walleye (*Stizostedion vitreum*) populations. Can. J. Fish. Aquat. Sci. 45: 643–654.

Mitochondrial DNA variation was examined in 141 walleye (*Stizostedion vitreum*) from 10 populations in the Great Lakes basin. Twenty-two hexanucleotide sequence recognition endonucleases were used, of which six (*Ava* I, *Bst* EII, *Cla* I, *Dra* I, *Nco* I, and *Sca* I) revealed polymorphisms between fish. Nine mitochondrial clones were resolved which fell into two major groups that differed in their *Nco* I and *Sca* I fragment patterns. The "A" group predominates in the eastern Great Lakes, while the "B" group predominates in the west. The geographical distribution of these two groups suggests that Great Lakes walleye persisted in at least two refugia during the last glaciation. The study revealed several rare mitochondrial genotypes which may prove useful in creating genetically marked brood stocks. Since mtDNA is maternally inherited, such markers would provide information on both the survival and long-term reproductive success of stocked fish.

Les auteurs ont étudié la variation de l'ADN mitochondrien chez 141 dorés jaunes (*Stizostedion vitreum*) de dix populations du bassin des Grands Lacs. Ils ont utilisé 22 endonucléases de reconnaissance de séquences d'hexanucléotides dont six (*Ava* I, *Bst* EII, *Cla* I, *Dra* I, *Nco* I et *Sca* I) ont montré l'existence d'un polymorphisme chez ces poissons. Neuf clones mitochondriens ont été décodés. Ils se répartissaient en deux grands groupes qui différaient quant aux fragments *Nco* I et *Sca* I. Le groupe « A » dominait dans la partie est des Grands Lacs, tandis que le groupe « B » dominait dans la partie ouest. La distribution géographique de ces deux groupes porte à croire que les dorés jaunes des Grands Lacs ont subsisté dans au moins deux refuges au cours de la dernière glaciation. L'étude a montré l'existence de plusieurs génotypes mitochondriens rares qui pourraient s'avérer utiles pour la création de stocks de géniteurs marqués génétiquement. Étant donné que l'ADNmt est hérité de la mère, de tels marqueurs pourraient fournir des renseignements sur la survie et le succès de la reproduction à long terme des poissons ensemencés.

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Walleye (*Stizostedion vitreum*) is one of the most economically important fish species in the Great Lakes (Regier et al. 1969; Scott and Crossman 1973; Schneider and Leach 1979). The biology and distribution of the species have been extensively studied (Regier et al. 1969; Scott and Crossman 1973; Spangler et al. 1977; Colby et al. 1979), largely with the aim of improving management of the species.

During the last half-century, Great Lakes walleye populations have declined drastically to the extent that in some areas, spawning stocks were lost (Regier et al. 1969; Schneider and Leach 1979). Since the mid-1970's, however, walleye densities have increased dramatically in the central Great Lakes (Hatch et al. 1987), while populations in other areas, such as Lake Ontario, are recovering more slowly (B. Malone, OMNR (Glenora), pers. comm.). It is not known whether these population changes have affected the genetic diversity of Great Lakes walleye. Recent recoveries in walleye populations might be due to the survival of few, or possibly even single, stocks of fish. Moreover, as Great Lakes walleye are managed using a basin-wide approach (e.g. Shuter et al. 1979), or according to political boundary criteria, further loss of individual stocks is likely as a result of inappropriate management.

Discrimination of walleye spawning stocks has relied almost entirely on tagging studies (Ferguson and Derksen 1971; Wolfert and Van Meter 1978) because allozyme studies on the Great Lakes populations have failed to reveal substantial differentiation in local gene frequency (Ulrikson and Laarman 1971; Colby and Nepszy 1981). Attempts to differentiate stocks using scale patterns (Riley and Carline 1982) or other morphological and physiological characters have also proved ineffective (Colby and Nepsy 1981).

The study of mitochondrial DNA (mtDNA) variation has several advantages over traditional methods in the examination of genetic diversity among conspecific individuals (Avise et al. 1979a, 1979b, 1984). Animal mtDNA is a circular molecule approximately 17 000 base pairs (bp) in length and is present in high copy number per cell, making it relatively easy to purify (Lansman et al. 1981). Restriction endonuclease digestion of mtDNA allows variation at the nucleotide level to be examined. Recent studies have demonstrated the occurrence of abundant mtDNA variation in freshwater fish (Avise et al. 1984; Berg and Ferris 1984; G. Wilson et al. 1985; Bermingham and Avise 1986; Thomas et al. 1986). The rate of nucleotide substitutions in mtDNA in higher vertebrates is approximately 10 times that

of the nuclear genome (Brown et al. 1979), further enhancing the resolution capability of mitochondrial DNA studies.

The strict maternal inheritance of mtDNA (Hutchinson et al. 1974; Gyllensten et al. 1985) may prove particularly useful in the context of fish population studies. In philopatric fish species, such as walleye, this has an important effect on the anticipated level of genetic divergence between stocks. The extent of genetic divergence between stocks is determined by the interaction between processes such as natural selection and genetic drift which promote divergence and gene exchange which reduces divergence. When populations are reasonably large and selection pressures weak, the amount of gene exchange is critical in determining the extent of divergence. For nuclear genes, this exchange can be accomplished by the movement of individuals of either sex. Thus, in the case where females are philopatric and males mate at several sites, no divergence in the nuclear genome will occur. However, this same pattern of gene exchange permits divergence of the mitochondrial genome. Males mating with females from different stocks will not prevent the divergence of the mitochondrial genome because of its maternal mode of inheritance. It is clear that the males of many fish species are more promiscuous than females. In walleye, females appear to return to the same spawning site year after year (Regier et al. 1969; Spangler et al. 1977), while tagged males have shown up in different spawning rivers in the same season (R. Haas, Michigan DNR (Mount Clemens), pers. comm.).

In summary, there are two main reasons to expect more clear-cut divergence among fish stocks in the mitochondrial rather than the nuclear portion of the genome: the more rapid rate of evolution of the mitochondrial genome and the failure of male exchange to reduce genetic divergence among stocks.

Studies on the mitochondrial versus nuclear genome also have a major advantage in the identification of prior episodes of gene pool fragmentation. During any period of isolation, divergence occurs in both the mitochondrial and nuclear genomes as a result of drift and selection. Initially, secondry contact between once isolated populations may be reflected in a steep cline of gene frequencies at nuclear loci. However, this divergence is ordinarily gradually eroded by gene flow, and in time, no indication of the period of isolation remains. By contrast, mitochondrial differences which evolved during a period of isolation may persist after fusion of the isolated populations because of the asexual transmission of the mitochondrial genome.

The ability to provide insight into colonization routes and past history of geographic isolation as well as the potential for stock discrimination provide dual incentives for mtDNA studies of fish populations. The present study has involved a survey of mtDNA diversity in walleye populations from 10 sites in the Great Lakes watershed. The results show both large amounts of mtDNA diversity and suggest colonization of the Great Lakes from at least two sources.

Methods

Collections

Nine populations of walleye were surveyed from within the Great Lakes as well as a population from Lake Simcoe (Table 1). All but two populations were collected at their spawning grounds.

Isolation of mtDNA

Approximately 5 g of liver from each fish was homogenized in 30 mL of cold sucrose (0.25 M) in STE buffer (10 mM NaCl; 10 mM Tris–HCl, pH 7.5; 1 mM EDTA, pH 8.0). The homogenate was centrifuged three times for 5 min at 1200 × *g* at 4°C to pellet cellular debris. The supernatant was then recentrifuged at 23 000 × *g* for 20 min at 4°C to pellet mitochondria. The supernatant was decanted and the pellet resuspended in 3.0 mL of TE buffer (0.01 M Tris–HCl, pH 7.6; 0.05 mM EDTA, pH 8.0) prior to its transferal to a sterile Falcon centrifuge tube (15 mL). Sodium dodecyl sulphate (SDS) (in H_2O) was added to give a final concentration of 1.0% SDS, and lysis was achieved by incubating the mixture for 10 min at 37°C (Bogenhagen and Clayton 1974). Once lysis was complete, indicated by a clearing of the suspension, one-sixth volume of TE, saturated with cesium chloride, was added and the tube placed at 4°C for a minimum of 1 h to precipitate cesium dodecyl sulphate, along with mitochondrial and other small cellular debris. Samples could be stored at this stage for up to 6 mo at 4°C without substantial reduction in the yield of mtDNA.

TABLE 1. Walleye populations collected for examination of mitochondrial DNA variation. Locations of each site are shown in Fig. 5.

Code	Site	Date sampled	No. of fish
1	Muskegon River (MUR)	April 1986	20[a]
2	Tittabawassee River (TIT)	April 1987	12[a]
3	Lake St. Clair, off Stoney Point (STC)	October 1985	7
4	Thames River (THA)	March–April 1985	33[a]
5	Western Lake Erie, off Colchester (ERC)	October 1985	7
6	Chick and Hen Island Reef, Lake Erie (ERW)	April 1985	23[a]
7	Lake Simcoe, Talbot River (SIM)	April 1986	10[a]
8	Trenton River, Bay of Quinte (TRE)	April 1986	8[a]
9	Napanee River, Bay of Quinte (NAP)	April 1986	12[a]
10	Lake Ontario, Athol Bay (ONT)	April 1986	9[a]

[a]Spawning populations.

The mtDNA was separated from RNA, nuclear DNA, and any remaining protein by propidium iodide–CsCl density gradient centrifugation. The tubes containing the lysate were centrifuged for 20 min at 14 000 × *g* to pellet the mitochondrial and small cellular debris. One and a half millilitres of the supernatant was then mixed with cesium chloride (1.1 $g \cdot mL^{-1}$ of lysate) in a Seton polyallomar centrifuge tube (Densmore et al. 1985) and 0.5 mL of propidium iodide (10 $mg \cdot mL^{-1}$ solution in STE) was added. Propidium iodide was used in preference to ethidium bromide because the separation between the nuclear and mitochondrial bands is approximately 1.8 times greater using this salt (Hudson et al. 1964). The density of this solution was adjusted to 1.57 $g \cdot mL^{-1}$ with cesium chloride (+) or TE (−) and the mixture overlain with light paraffin oil to prevent tube collapse during centrifugation.

A density gradient was established by 48–60 h of centrifugation at 36 000 rpm and 20°C in a Beckman L5-65 ultracentrifuge using a SW60 Ti rotor. Following centrifugation, nucleic acids were visualized by long-wave (approximately 305 nm) ultraviolet-induced fluorescence. The band of closed circular mtDNA (identified by its position approximately 5 mm below a variable-sized band, containing mainly nuclear DNA, in the upper portion of the centrifuge tube) was withdrawn by bottom puncture using a 0.7-mm-diameter needle (see Lansman et al.

TABLE 2. Fragment sizes (kilobase pairs) of walleye mtDNA for those restrictions enzymes revealing polymorphisms or cutting more than once. Fragment pattern types and total length of molecule, as determined for each enzyme, are also given.

Enzyme	Pattern	Fragment size	Total length
Apa I	A	11.30,3.19,2.38	16.87
Ava I	A	5.36,3.05,2.70,2.12,1.01, 0.98,0.92,0.22,0.20,0.17, 0.14,0.11,0.06	17.04
	B	5.36,3.05,2.90,2.12,1.01, 0.98,0.92,0.22,0.20,0.14, 0.11,0.06	17.07
	C	5.36,2.80,2.70,2.12,1.01, 0.98,0.92,0.25,0.22,0.20, 0.17,0.14,0.11,0.06	17.04
Bam HI	A	16.60,0.31	16.91
Bcl I	A	8.18,5.74,2.31,0.83	17.06
Bst EII	A	16.90	16.90
	B	9.89,7.05	16.94
	C	9.72,7.18	16.90
Dra I	A	11.35,4.12,1.51	16.98
	B	11.35,5.63	16.98
	C	7.48,4.12,3.87,1.51	16.98
Eco RV	A	8.87,5.60,2.10,0.11	16.68
Hind III	A	9.91,2.35,1.80,1.70,0.98	16.74
Nco I	A	9.84,5.16,0.84,0.73	16.57
	B	10.60,5.16,0.84	16.60
Pst I	A	8.15,5.92,2.73	16.80
Sal I	A	13.97,2.87	16.84
Sca I	A	8.82,4.48,1.90,1.42	16.62
	B	4.77,4.48,4.05,1.90,1.42	16.62
Sma I	A	13.40,3.14,0.14	16.68
Sst II	A	14.86,1.75,0.26	16.87
Xba I	A	9.65,7.34	16.99
Xho I	A	11.81,5.15	16.96
Mean length (± SD)(n = 24)			16.86 ± 0.16

1981). Ribonucleic acids formed a diffuse band in the lower portion of the centrifuge tube, but did not interfere with the extraction. On average, 300 μL of solution was withdrawn from each tube. When a clear mtDNA band was absent, 400 μL of solution was collected from the region in which the mtDNA band was normally visualized. Up to 500 ng of mtDNA could be recovered from these samples.

The propidium iodide was removed from the sample by three to four extractions with 800 μL of *n*-butanol (saturated with water and NaCl) until all the pink colour was removed. Samples were then dialysed for a minimum of 12 h against a salt dialysis solution (1 M NaCl; 10 mM Tris–HCl, pH 8.0; 1 mM EDTA, pH 8.0) and then twice for 12 h against a salt-free dialysis solution (10 mM Tris-HCL, pH 8.0; 1 mM EDTA, pH 8.0). Purified mtDNA was frozen (−20°C) until required for restriction analysis.

Yields of mtDNA ranged between 1500 and 3000 ng (in 200–400 μL of aqueous solution) per 4–5 g of liver tissue sample, with the highest yields obtained from fish collected during the spawning season. mtDNA was normally extracted to the lysis stage within 24 h of death, but extractions were successful for up to 96 h, if livers were stored on ice.

Restriction Endonuclease Digestion

The purified mtDNA was digested with restriction endonucleases, obtained from Bethesda Research Laboratories and Boehringer Mannheim, employing recommended temperature and buffer conditions. A 2-h incubation period was usually sufficient to achieve complete digestion of 10 ng of walleye mtDNA in a 15-μL volume of digestion mixture, containing 1.3 units of enzyme. All fish were surveyed using nine six-base sequence recognition endonucleases: *Ava* I (C/PyCGPuG), *Bcl* I (T/GATCA), *Bst* EII (G/GTNACC), *Cla* I (AT/CGAT), *Dra* I (TTT/AAA), *Eco* RI (G/AATTC), *Hind* III (A/AGCTT), *Nco* I (C/CATGG), and *Sca* I (AGT/ACT). A subset of 65 walleye (18 THA, 13 ERW, 7 ERC, 6 STC, 6 MUR, and 3 fish from each of the other five sites, Table 1) were surveyed for an additional 13 endonucleases: *Apa* I (GGGCC/C), *Bam* HI (G/GATCC), *Bgl* II (A/GATCT), *Eco* RV (GAT/ATC), *Kpn* I

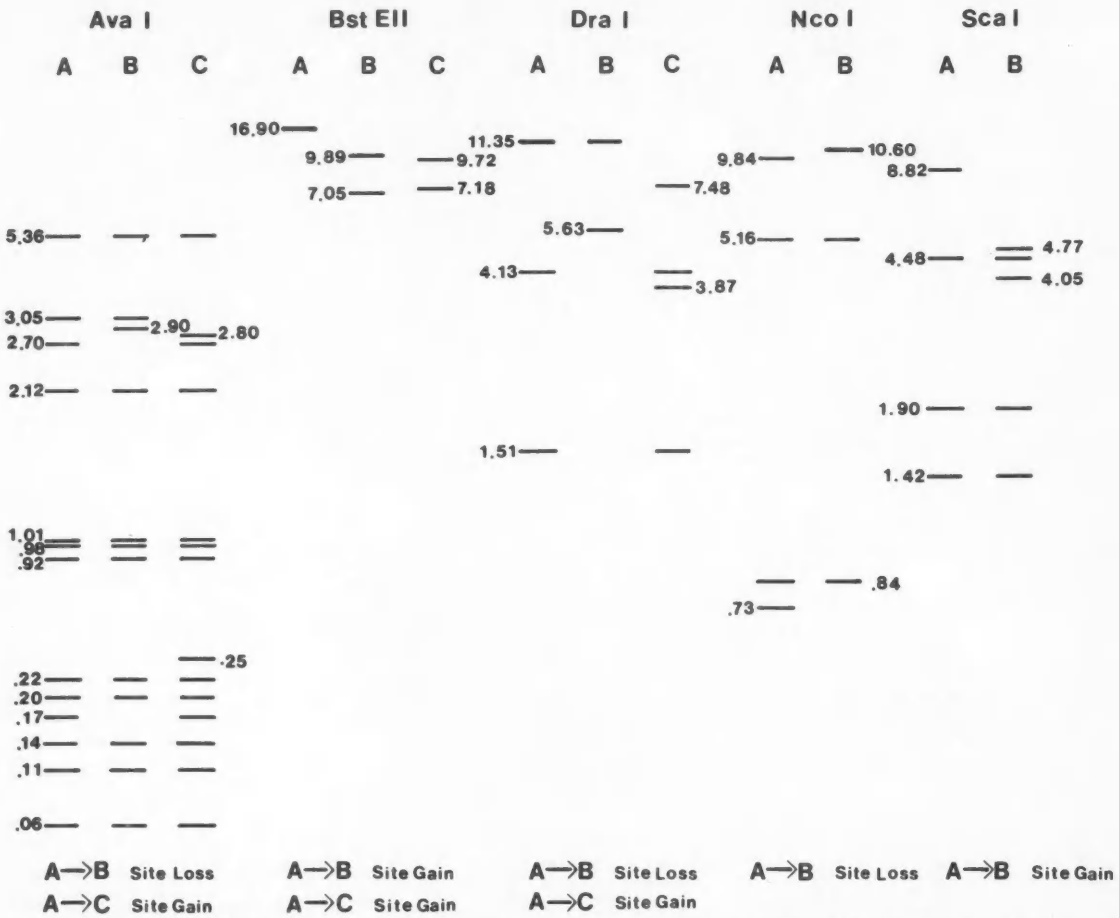

FIG. 1. Polymorphic restriction fragment patterns observed in walleye mtDNA and the most probable path by which each pattern has been derived. Fragment sizes in kilobase pairs.

(GGTAC/C), *Mlu* I (A/CGCGT), *Pst* I (CTGCA/G), *Pvu* I (GCAT/CG), *Pvu* II (CAG/CTG), *Sal* I (G/TCGAC), *Sst* I (GAGCT/C), *Sst* II (CCGC/GG), and *Xba* I (T/CTAGA).

Radioactive End-Labelling, Gel Electrophoresis, and Autoradiography

After digestion was complete, the restriction fragments were end-labelled with ^{32}P-labelled nucleotides using a procedure modified with Brown (1980). The end-labelling mixture consisted of 0.6 unit per sample of *Escherichia coli* DNA polymerase I large (Klenow) fragment (Bethesda Research Laboratories), with 4 μL of its buffer (7 mM β-mercaptoethanol; 6 mM KCl; 10 mM $MgCl_2$; 10 mM Tris–HCl, pH 7.5; T. Dowling and W. M. Brown, pers. comm.), 22.2 kBq each of [α-^{32}P]dATP and [α-^{32}P]dCTP (Amersham; 111 TBq·mmol^{-1}, 370 MBq·mL^{-1}) and 0.5 μL each of unlabelled dGTP and dTTP (each 2 mM in 50 mM Tris–HCl, pH 7.5). In practice, enough of this mixture for a set of 16 digests was made up and 5 μL aliquots dispensed into each tube containing restricted DNA. The tubes were then incubated at 4°C for 30–45 min.

Three hundred microlitres of 0.3 M sodium acetate (pH 7.8) containing 30 μg of yeast tRNA (Sigma) was added and nucleic acids precipitated by the addition of 1 mL of cold (−20°C) absolute ethanol, followed by incubation at −70°C for a minimum of 1 h, but usually overnight. After centrifugation for 20 min at 4°C, the supernatant was decanted and 1 mL of cold (−20°C) 70% ethanol was added. The pellet was gently rinsed by inverting the tube five to six times, following which the tube was recentrifuged for 10 min. The ethanol was decanted off once more and the pellet dried under vacuum for 1 h at room temperature. Once dry, the pellet was vigorously resuspended in 15 μL of loading buffer (8% sucrose; 0.05% bromophenol blue; in 1× TBE). Half of this mixture was then loaded into an agarose gel and the other half into a polyacrylamide gel for electrophoresis.

Electrophoresis of DNA fragments was performed in horizontal 1.0 or 1.2% agarose gels (17 × 14 × 1.5 mm) and 4% polyacrylamide (19:1 acrylamide:bisacrylamide) (42 × 14 × 0.75 mm) in 1× TBE buffer (89 mM Tris; 89 mM boric acid; 2mM EDTA, pH 8.0). Fragments were run into gels for 5 min at high voltage (200–300 V) and then electrophoresed at 34 V (2 V·cm^{-1}) in agarose and 150 V (3.6 V·cm^{-1}) in acrylamide gels for approximately 12 h at room temperature.

TABLE 3. Fragment sizes (kilobase pairs) of walleye THA32 mtDNA produced by restriction endonuclease digestion. Total length of molecule and size increases relative to usual length are also given. Fragments showing increase are underlined.

Enzyme	Fragment size	Total length	Increase
Ava I	5.36,4.51[a],3.05,2.12,1.01, 0.98,0.92,0.22,0.20,0.17, 0.14,0.11,0.06	18.85	1.81
Bcl I	9.82,5.74,2.31,0.83	18.70	1.64
Dra I	11.35,7.28[b]	18.63	1.65
Eco RV	8.84,5.61,3.94,0.11	18.50	1.82
Hind III	11.57,2.35,1.80,1.70,0.98	18.40	1.66
Nco I	12.33,5.16,0.84	18.33	1.74
Pst I	9.82,5.92,2.73	18.47	1.67
Sca I	4.80,4.48,4.10,1.90,1.67[c], 1.42	18.37	1.75
Sma I	13.3,5.04,0.14	18.48	1.80
Xba I	9.65,8.86	18.51	1.52
Xho I	11.81,6.91	18.72	1.73
Mean length (± SD) ($n = 11$)		18.54 ± 0.16	1.71 ± 0.09

[a] *Ava* I increase is on 2.70-kb fragment.
[b] THA32 shows a *Dra* I "B" pattern, with increase on the 5.63-kb fragment.
[c] *Sca* I increase appears as an extra 1.67-kb fragment, with a small (0.05 kb) increase on the 4.05-kb fragment.

Once electrophoresis was complete, the gels were dried onto Whatman 3MM filter paper, under reduced pressure at 80°C, using a Bio-Rad 483 slab dryer. The filter-bound gels were then exposed to Fuji RX film for autoradiography, either at room temperature, or using Dupont Cronex Hi-plus intensifying screens at −70°C, for between 6–48 h depending on the level of radioactivity.

DNA Size Estimation

The sizes of mtDNA restriction fragments were estimated with reference to the mobilities of pBR322 (cut with *Hpa* II) standards on the acrylamide gels and lambda-DNA standards on the agarose gels. The lambda-DNA standard used comprised a mixture of lambda-DNA cut with *Hind* III and lambda-DNA double digested with *Eco* RI and *Hind* III. The fragment sizes of the standards were determined from the sequence data reported by Sanger et al. (1982) for phage lambda-DNA and Maniatis et al. (1982) for pBR322. Fragments were sized directly from the autoradiographs using the program DNAGEL (Kieser 1984) as modified by P. Grewe (Department of Biological Science, University of Windsor, unpubl., details available on request) with an APPLE II+ microcomputer and a HOUSTON graphics tablet.

Analysis of Walleye mtDNA Fragment Patterns

Polymorphic restriction fragment patterns were labelled with respect to the endonuclease used and in the order that the fragment pattern was discovered (i.e. *Nco* I "A," "B," etc.). When the restriction fragment patterns were monomorphic for a particular endonuclease, only an "A"-type pattern was designated. Each fish was allotted a series of letters representing the mtDNA fragment pattern observed for each enzyme, producing a composite pattern for each fish. Fish with the same composite pattern were classed as members of the same mitochondrial clone. The number of restriction sites for each clone and the number of shared sites between clones were then calculated. The homology of all patterns was checked by conducting double digests and by mapping the walleye mtDNA molecule for both the monomorphic and polymorphic cut sites.

The maximum likelihood estimate of the number of nucleotide differences per site (d) was calculated using the methods of Nei et al. (1985). This entails comparing the number of restriction sites m_x and m_y (for mtDNA molecules x and y, respectively) with m_{xy}, the number of shared restriction sites. Values of d were then used to construct an UPGMA phenogram (Nei et al. 1985).

Restriction Map of Walleye mtDNA

A restriction site map of the walleye mtDNA molecule was constructed for 24 hexanucleotide recognition sequence endonucleases by conducting a series of single and double digests (Lawn et al. 1978; Maniatis et al. 1982). Double digests conducted using an endonuclease producing a blunt-ended cut, along with one producing a sticky-ended cut, greatly simplified the mapping procedure, as fragments produced by each type of enzyme could be easily identified (N. Billington, pers. obs.). In this manner, the relative ordering of the cut sites with respect to each other was determined. In addition to the 22 endonucleases employed in the population survey, *Sma* I (CCC/GGG) and *Xho* I (C/TCGAG) were used to facilitate mapping the large number of *Ava* I sites, as two of the four sequences recognized by *Ava* I are individually recognized by these enzymes.

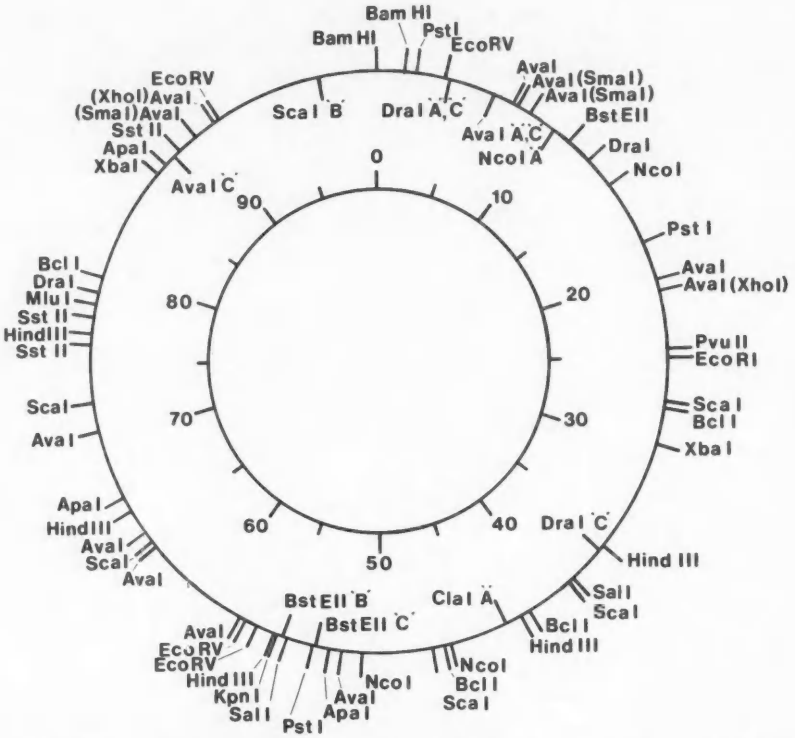

FIG. 2. Restriction map of walleye mtDNA for 24 endonucleases. Invariant sites are mapped on the exterior and variable sites on the interior of the circle. A *Bam* HI site was used as the origin of the map. Letters associated with variable sites refer to the particular polymorphic patterns obtained. Interior circle shows map units, based on an arbitary scale of 0–100.

TABLE 4. Walleye mitochondrial DNA clones identified using six polymorphic endonucleases.

	Endonuclease pattern type						
Clone	*Nco* I	*Sca* I	*Dra* I	*Bst* EII	*Ava* I	*Cla* I	Fish
1	A	A	A	A	A	O	44 fish
2	A	A	C	A	A	O	ERW8
3	B	A	A	B	A	O	THA31, TRE6, NAP7
4	B	B	A	A	A	O	84 fish
5	B	B	A	C	A	O	ERW7, ERW16
6	B	B	A	A	B	O	STC1
7	B	B	A	A	C	O	ERC3
8	B	B	B	A	A	O	THA17, ERW6, ERW18
9	B	B	A	A	A	A	THA16, ERW13

Results

Restriction Fragment Patterns and Length of Walleye mtDNA Molecule

Twenty-one of the 24 endonucleases cut walleye mtDNA at least once; sites were absent for *Bgl* II, *Pvu* I, and *Sst* I. Four endonucleases (*Eco* RI, *Kpn* I, *Mlu* I, and *Pvu* II) cut walleye mtDNA once and a single *Cla* I site was found in two fish, but the mtDNA of other walleye lacked this site. Five other endonucleases (*Ava* I, *Bst* EII, *Dra* I, *Nco* I, and *Sca* I) revealed polymorphic restriction fragment patterns (Table 2). Each fragment pattern change could be explained by a single site gain or loss (Fig. 1).

Sixteen restriction endonucleases cut walleye mtDNA more than once (Table 2). The length estimates for the walleye mtDNA molecule based on these results averaged 16 860 ± 160 bp. One Thames River fish (THA32) had a mitochondrial genome which was approximately 1.7 kilobases (kb) longer than normal (Table 3). This length increase was due to a single insert, as there was a length increase of this magnitude in only one fragment for each endonuclease used, except for *Sca* I. This endonuclease revealed an additional 1.67-kb fragment inserted between the *Sca* I "B" site at map position 97 (Fig. 2) and the adjacent 4.05-kb fragment (THA32 showed a *Sca*I "B" pattern otherwise); this latter fragment also showed a small (0.05 kb) length increase. A similar insert (1.62 ± 0.11 kb long) was

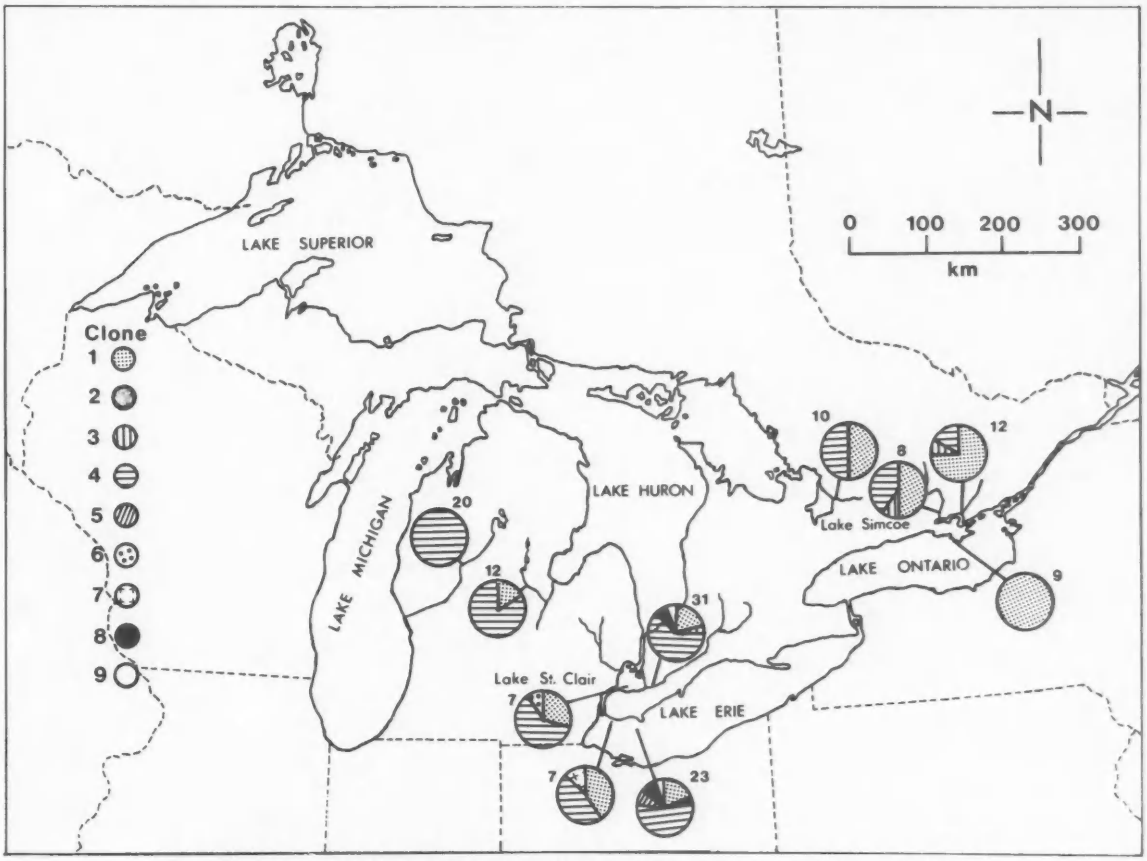

FIG. 3. Distribution of walleye mtDNA clones. Numbers of fish examined at each site are also shown.

TABLE 5. Matrix of transformed distances ($d \times 10^{-3}$) between clones (above diagonal) and numbers of shared restriction sites between clones used to generate these distances (below diagonal) for nine Great Lakes walleye mitochondrial clones.

	1	2	3	4	5	6	7	8	9
1		1.46	2.95	2.95	4.41	4.49	4.41	4.49	4.41
	57								
2			4.41	4.41	5.85	5.95	5.85	5.95	5.85
	57	58							
3				2.95	4.41	4.49	4.41	4.49	4.41
	56	56	57						
4					1.46	1.48	1.46	1.48	1.46
	56	56	56	57					
5						2.95	2.90	2.95	2.99
	56	56	56	57	58				
6							2.95	3.00	2.95
	55	55	55	56	56	56			
7								2.95	2.90
	56	56	56	57	57	56	58		
8									2.95
	55	55	55	56	56	55	56	56	
9									
	56	56	56	57	57	56	57	56	58

NOTE: Numbers of shared restriction fragments between clones based on data obtained using 22 six-base restriction endonucleases.

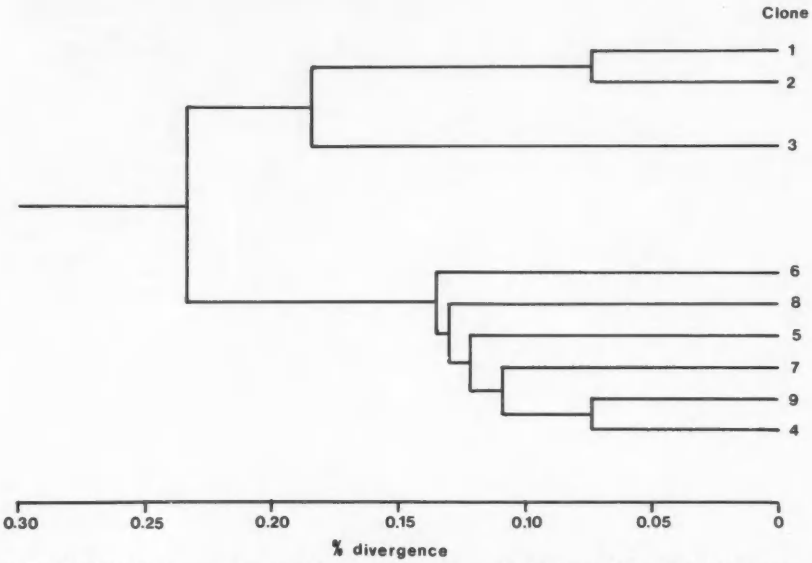

FIG. 4. UPGMA phenogram of percent divergence for walleye mtDNA clones. Note that % divergence is plotted as $0.5 \times d$, after Nei et al. (1985).

also found in the same part of the mtDNA molecule of a second Thames River fish (THA22). However, the mtDNA of this latter fish contained a number of additional restriction sites not found in the other fish examined; these additional sites were not mapped in the present study. These fish were excluded from genetic distance analyses. No other size variation was detected and there was no evidence of heteroplasmy.

Between 54 and 63 restriction sites were resolved with the 24 endonucleases employed in this study. Figure 2 shows the position of 54 invariant and 9 variable sites on the molecule. Thus, 324–378 nucleotides representing 1.92–2.24% of the genome were surveyed. On average, each endonuclease cut the walleye mtDNA molecule 2.16–2.52 times (if *Xho* I and *Sma* I are not included in the calculations, effectively 25 endonucleases were used, as *Ava* I recognizes four sequences).

Mitochondrial DNA Diversity and Geographical Patterns of mtDNA Variation

Nine mitochondrial clones were resolved based on the composite patterns identified using the five polymorphic endonucleases (Table 4). Two of the clones (clones 1 and 4) were very common (comprising 32.1 and 59.6% of all fish examined, respectively) and widely distributed (Fig. 3). The remaining clones were rare, being found in only one, two, or three fish.

To examine the relationships among clones, the restriction site similarities among clones were compiled (Table 5) and d, the maximum likelihood estimate of the number of nucleotide differences per site, was calculated. The number of restriction sites used to calculate d values was based on data obtained using 22 endonucleases on the subsample of 65 fish.

The UPGMA phenogram generated from these data (Fig. 4) indicated that the walleye mitochondrial clones were separable into two groups, designated A and B, with six of the nine clones belonging to this latter group. The two groups were separable on the basis of *Nco* I and *Sca* I fragment patterns. Specifically, most group A fish possessed an extra *Nco* I site (*Nco* I "A" pattern), which was not present in group B fish, and lacked a *Sca* I site (*Sca* I "A" pattern), present in group B. The three fish belonging to clone 3 represented an exception to this association, as they lacked both the *Nco* I and *Sca* I sites. Clone 3 was also exceptional in being the sole clone possessing the *Bst* EII "B" pattern.

The frequencies of the A and B groups (Fig. 5) showed a clear geographical pattern, with the B group predominating in the west and the A group in the east (the clone 3 fish were excluded from this analysis). Although some B group fish were found in the two Bay of Quinte populations, none of the 20 fish from the most westerly site (Muskegon River) belonged to the A group.

Discussion

The length estimate for the mtDNA molecule of most walleye agreed closely with estimates for other fish species (Table 6). However, two of the Thames River walleye had mtDNA molecules approximately 1.7 kb longer than those of the other individuals. There are prior reports of length variation in fish mtDNA molecules, although none involves such a large insert. Beckwitt and Petruska (1985) found considerable interspecific length variation in members of the family Scorpaenidae, while Bermingham et al. (1986) reported single inserts of up to 900 bp among bowfin (*Amia calva*). Brown (1983) reported mtDNA length differences of up to 1.7 kb between species of the lizard genus *Cnemidophorus*, and Densmore et al. (1985) showed that length variation of approximately 370 bp occurred frequently within *Cnemidophorus tesselatus*. These latter length variants were shown to be due to variability in the copy number of a tandemly repeated 64-bp sequence adjacent to the D-loop region (Densmore et al. 1985). More recently, Moritz and Brown (1986) found length increases of 4.65, 4.80, and 6.20 kb in *Cnemidophorus exsanguis* which were due to the tandem duplication of large sections of the molecule, including both the D-loop region and the adjacent transfer and ribosomal RNA genes.

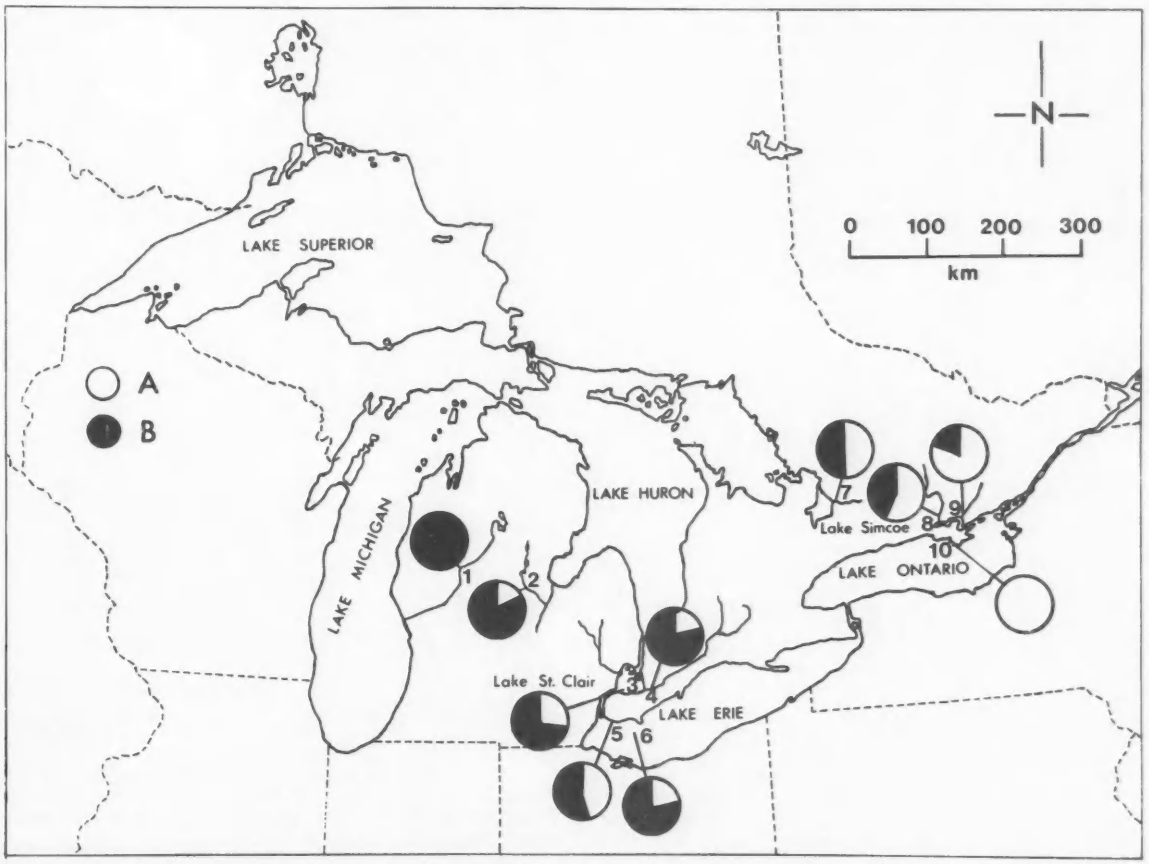

FIG. 5. Percentage occurence of the *Nco* I/*Sca* I "A" (open portion) and "B" (shaded portion) mtDNA patterns in the 10 walleye populations across the Great Lakes basin. The three clone 3 fish are excluded. Numbers refer to site code (Table 1).

The length increase in the walleye involved insertion of a single 1.7-kb section of DNA, in the vicinity of the D-loop region (N. Billington, P. D. N. Hebert, and R. D. Ward, unpubl. data) which would appear to be a tandem duplication as evidenced by the repeated *Sca* I site. Densmore et al. (1985) suggested that, as recombination is absent in the mitochondrial system of multicellular animals (Brown 1983), slipped mispairing during replication (Streisinger et al. 1966) is a likely mechanism by which length variation within a species may arise.

Mitochondrial DNA Variation within Spawning Populations

The use of six base endonucleases allowed the resolution of nine walleye clones within the central and eastern Great Lakes area, with two clones comprising most of the fish sampled. The greatest clonal diversity occured in walleye from Lake St. Clair and the western basin of Lake Erie. However, none of the clones was restricted to fish from a particular spawning site, so it was impossible to discriminate between individual spawning stocks.

Management Implications of Walleye mtDNA Variation

The existence of rare mtDNA variants has important management implications. Large numbers of one clone could be reared in hatcheries and then introduced into stocking programs. A diverse nuclear genome could be maintained by breeding males from a variety of stocks with females from the selected clone. As mtDNA is maternally inherited, all the offspring would inherit the mtDNA pattern of their mother. Both the fate of these introduced fish and their breeding success could be determined for many generations by monitoring the frequency of the rare mtDNA type. For example, in the present study only 1 of the 141 fish showed the *Dra* I "C" pattern. If females with this genotype were used as the founders of a brood stock, they could be discriminated from wild fish with >99% confidence. By isolating a brood stock with two or three such markers, fish belonging to it would be distinctive in virtually all cases. Such mitochondrially marked fish would be far more useful for stocking programs than fish with variant allozyme markers. Although allozyme markers are useful for following single cohorts of introduced fish (Ward and Clayton 1975; Murphy et al. 1983), genotypic frequencies return to Hardy–Weinberg equilibrium after a single generation.

Evolution of Central Great Lakes Walleye mtDNA Variation

The genetic distance analysis revealed that the nine mitochondrial clones split into two main groups. Genetic distance values (d) between the nine clones ranged from 0.0015 to 0.0060, values comparable with those reported for other fish

TABLE 6. Reported lengths for mitochondrial DNA molecules in fish species.

Species	Size (bp)	Reference
Lepomis macrochirus	16 200	Avise et al. 1984
Katsuwonus pelamis	16 900	Graves et al. 1984
Oncorhynchus tshawytscha	16 670	Berg and Ferris 1984
Salmo gairdneri	16 670	
Salmo trutta	16 670	
Salvelinus fontinalis	16 670	
Scorpaena guttata	19 500 ± 300	Beckwitt and Petruska 1985
Sebastes atrovirens	17 300 ± 400	
Sebastes caurinus	17 400 ± 400	
Sebastes melanostomus	17 200 ± 400	
Sebastes m[illegible]inus	16 900 ± 400	
Scomber japonicus	17 200 ± 400	
Salmo salar	16 700	Birt et al. 1986
Oncorhynchus kisutch	16 500 ± 500	Thomas et al. 1986
Oncorhynchus tshawytscha	16 500 ± 500	
Oncorhynchus nerka	16 500 ± 500	
Oncorhynchus gorbuscha	16 500 ± 500	
Oncorhynchus keta	16 500 ± 500	
Salmo gairdneri	16 500 ± 500	
Crassius auratus	16 100 ± 220	Beckwitt and Aoyagi 1987
Salvelinus namaycush	16 800 ± 200	Grewe and Hebert 1987
Stizostedion canadense	16 700 ± 260	Billington and Hebert 1986
Stizostedion lucioperca	16 740 ± 280	
Stizostedion vitreum	16 860 ± 160	Present study
Stizostedion vitreum (THA22)	18 520 ± 110	
Stizostedion vitreum (THA32)	18 540 ± 160	

species. For example, G. Wilson et al. (1985) reported a range of 0.001–0.011 for intraspecific mtDNA sequence divergence among rainbow trout (*Salmo gairdneri*) stocks and values of approximately 0.0024 among chum salmon (*Oncorhynchus keta*) stocks. Thomas et al. (1986) found values ranging from 0.0016 in sockeye (*O. nerka*) and coho salmon (*O. kisutch*) to 0.0045 between rainbow trout populations. Bermingham and Avise (1986) summarized intraspecific d values in *Amia calva* and four species of *Lepomis*, with d values for *Amia* ranging from 0.002 to 0.012 and those for *Lepomis punctatus*, *L. microlophus*, and *L. gulosus* ranging between 0.002 and 0.081, 0.002 and 0.100, and 0.001 and 0.077, respectively; the d value for *Lepomis macrochirus* was 0.085 (Avise et al. 1984; Bermingham and Avise 1986). Most of the larger d values between *Lepomis* mitochondrial clones were related to geographic isolation dating to the Pleistocene interglacial (Bermingham and Avise 1986).

Some species show very low levels of intraspecific divergence. For example, Graves et al. (1984) found no variation between Atlantic and Pacific stocks of the skipjack tuna (*Katsuwonus pelamis*), and a lack of mtDNA divergence was also reported between anadromous and nonanadromous populations of Atlantic salmon (*Salmo salar*) (Birt et al. 1986). Intraspecific differences in populations of the American eeel (*Anguilla rostrata*) were very low ($d < 0.001$), much lower, in fact, than between populations of the European eel (*A. anguilla*) ($d = 0.008$) (Avise et al. 1986).

The level of mtDNA sequence divergence among congeneric fish species is usually at least an order of magnitude greater than intraspecific variation, ranging from 0.02 to 0.10. Thus, Thomas et al. (1986) reported d values of 0.025–0.069 among five *Oncorhynchus* species. A similar range of $d = 0.034$–0.072 was also reported between salmonids by Berg and Ferris (1984) and $d = 0.037$ between the American and European eels (Avise et al. 1986).

Brown et al. (1979) determined the rate of mtDNA sequence divergence for several mammalian species (mainly primates) which, when averaged over the whole molecule, was linear for species that separated less than 8–10 million yr ago and was approximately 2% per million yr. Other studies involving vertebrate species generally corroborate this value (A. Wilson et al. 1985, and references cited therein), which has been used by some workers to estimate divergence times for salmonids (G. Wilson et al. 1985; Thomas et al. 1986) and other species of fish (Bermingham and Avise 1986). Assuming that the molecular clock calibration of Brown et al. (1979) can be applied to percid mtDNA, the 0.467 ± 0.200% divergence between the two main groups of walleye suggests a period of approximately 230 000 ± 100 000 yr since they shared a common ancestor. This represents the Sangamon interglacial period prior to the Wisconsin glacial advance during the Pleistocene (Prest 1976), and it seems possible that the two groups observed in central Great Lakes walleye may reflect the result of colonization from separate glacial refugia. The east–west shifts in the relative frequencies of the two groups suggest that group A rose to dominance in populations from an Atlantic refugium, while group B was associated with fish from the Mississippi refugium.

Radforth (1944) suggested that the Great Lakes were colonized by walleye from both Atlantic and Mississippi refugia, based on an analysis of the current distribution of the species, in relation to the time and place of known barriers and connections. However, Bailey and Smith (1981), using the same approach, concluded that walleye colonized the Great Lakes solely from the Mississippi refugium. Furthermore, they suggested that current Great Lakes stocks stem from differentiation within the basin, or reflect interglacial events that occurred in Mississippi refugia.

Electrophoretic studies of a polymorphic muscle protein show some evidence of clinal shifts in gene frequency across Canadian walleye populations. Uthe et al. (1966) found that fish from Great Slave Lake were homozygous at a muscle protein locus, which was polymorphic in Lake St. Clair fish. Further studies (Uthe and Ryder 1970) showed a regional variation in the distribution of this muscle myogen polymorphism, with the A allele being most common in western sites (Great Slave Lake and Lake Winnipeg), while the B allele was more common in central and eastern Lakes. A few heterozygotes were found in Lake Winnipeg, but they were much more common in the central Great Lakes region. Uthe and Ryder (1970) concluded that this geographic variation was consistent with the hypothesis of colonization from two refugia.

The mtDNA analysis provides more tangible support for this hypothesis. However, distributional patterns of mitochondrial types in the Great Lakes may have been clouded by the intensive stocking programs conducted between 1890–1930 which involved transfer of fish between the lakes (Regier et al. 1969). More detailed information on postglacial dispersal of walleye can best be gained by supplementing work on the Great Lakes with studies on inland lakes containing only natural populations.

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Hydrologic and Riparian Influences on the Import and Storage of Coarse Particulate Organic Matter in a Prairie Stream

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The hydrologic regime and zonation of riparian vegetation influenced the quantity and quality of coarse particulate organic matter (CPOM; >1 mm) stored in the channel and upper bank of a prairie stream. In a 5.4-km intermittent reach of the South Branch of Kings Creek on Konza Prairie, Kansas, total annual import was lowest in headwater reaches and increased downstream. Total storage of benthic CPOM in the dry channel and on the bank before the flow period was highest in the fourth- and fifth-order gallery forest zone (999 g ash-free dry mass·m^{-2}) and less in upstream reaches (320–341 g·m^{-2}). These longitudinal patterns of CPOM annual import and storage (before the flow period) were opposite those predicted by the river continuum concept for streams draining forested regions. Following flow, headwater channels had more CPOM (291 g·m^{-2}) than downstream reaches. On the bank, storage was always highest in downstream reaches. Composition of CPOM both in the channel and on the bank varied with changes in riparian vegetation; grass tissues dominated in headwater channels, while wood and leaves of trees and shrubs were more abundant downstream. During the flow period, storage of CPOM increased only in headwater channels, where retention was high despite the lack of woody debris. In this intermittent prairie stream, benthic CPOM may not contribute consistently to the terrestrial/aquatic linkages that are suggested in the river continuum concept because of (1) a paucity of large CPOM sources (e.g. trees, shrubs) in the upper reaches and (2) a hydrologic regime that reduces the amount, as well as the predictability, of stored CPOM. The biota of prairie streams must have opportunistic food gathering and reproductive strategies to take advantage of variable food resources in a flow environment that is itself very unpredictable.

Le régime hydrologique et la répartition zonale de la végétation riveraine influaient sur la quantité et la qualité de la matière organique particulaire grossière (MOPG; > 1 mm) emmagasinée dans le chenal et la rive supérieure d'un cours d'eau de prairie. Dans une section de 5,4 km à écoulement intermittent de la branche sud du ruisseau Kings de Konza Prairie (Kansas), l'apport annuel total était le moins important dans les parties d'amont et augmentait en direction de l'aval. L'emmagasinement total de MOPG benthique dans le chenal à sec et sur la rive avant la période d'écoulement était le plus élevé dans la zone forestière en galerie d'ordres quatre et cinq (999 g de masse sèche sans cendre·m^{-2}) et moins important dans les sections d'amont (320–341 g·m^{-2}). Ces allures longitudinales de l'apport et de l'emmagasinement annuel de la MOPG (avant la période d'écoulement) étaient contraires à celles prévues par le concept du continuum des cours d'eau pour les cours d'eau drainant des régions forestières. Après l'écoulement, la teneur en MOPG des chenaux d'amont (291 g·m^{-2}) était supérieure à celle des sections d'aval. Sur les rives, l'emmagasinement était toujours plus important dans les sections d'aval. La composition de la MOPG du chenal et des rives variait en fonction de la végétation riveraine; les tissus de plantes herbacées dominaient les chenaux d'amont tandis que ceux du bois et des feuilles d'arbres et d'arbustes étaient plus abondants en aval. Au cours de la période d'écoulement, l'emmagasinement de la MOPG n'augmentait que dans les chenaux d'amont où la rétention était importante, ceci en dépit de l'absence de débris ligneux. Il est possible que, dans ce cours d'eau de prairie à écoulement intermittent, la MOPG benthique ne contribue pas de façon constante aux liaisons terrestres/aquatiques que suppose le concept du continuum des cours d'eau à cause de : 1) la pauvreté des importantes sources de MOPG (p. ex. : arbres et arbustes) dans les sections d'amont, et 2) un régime hydrologique qui réduit la quantité de la MOPG emmagasinée de même que la capacité de la prévoir. Le biote des cours d'eau de prairie se doit de posséder des stratégies opportunistes d'alimentation et de reproduction afin de tirer avantage de ressources alimentaires variables dans un environnement en écoulement lui-même très imprévisible.

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Benthic organic matter provides food and habitat for stream biota and enhances retention characteristics of the stream channel, regulating export of dissolved and particulate materials to downstream reaches (Bilby and Likens 1980; Speaker et al. 1984). Detritus accumulations, particularly in woody debris dams, are important habitat features for stream microflora, invertebrates, and fishes (e.g. Angermeier and Karr 1984; Benke et al. 1984). However, both spatial and temporal sampling problems have made storage one of the most poorly documented components of organic matter budgets (Cummins et al. 1983).

The river continuum concept suggests that, in a hypothetical stream with headwaters draining a forested catchment, storage of organic matter should be highest in headwater reaches and decrease in a downstream direction, with a shift in particle size from coarse to finer particles (Vannote et al. 1980). This concept also suggests that the degree of influence of riparian vegetation changes in a regular fashion along a downstream sequence; longitudinal changes in riparian vegetation are expected to affect the quantity and species composition of organic matter that enters the stream as well as its biological fate (Conners and Naiman 1984; Cummins et al. 1984). We expected intermittent prairie streams to have a different longitudinal pattern of import and storage than the generalized river continuum stream because headwater channels are bordered by riparian grasses, while downstream reaches are bordered by a gallery forest of deciduous trees (Gurtz et al. 1982).

In addition, streams that drain tallgrass prairie are subject to frequent hydrologic extremes of flooding and drought. Recent flow history affects decomposition rates (Gurtz and Tate 1988) and storage (Cummins et al. 1983) of organic matter; thus, we expected large temporal variations in organic matter storage.

This paper investigates the (1) quantity and species composition of coarse particulate organic matter (CPOM) imported via direct and lateral mechanisms along a longitudinal gradient of an intermittent prairie stream, (2) quantity and composition of CPOM stored in three different riparian zones before and after a summer flow period, (3) net loss of CPOM from the catchment during that flow period, and (4) relative storage of CPOM in the channel versus the bank and any redistribution that occurred during the flow period.

Site Description

Kings Creek lies within the 3487-ha Konza Prairie Research Natural Area, a tract of native tallgrass prairie owned by The Nature Conservancy near Manhattan, Kansas (Fig. 1). Konza Prairie is representative of the Flint Hills Upland, which extends from Oklahoma north through Kansas. It is a dissected upland with hard chert- and flint-bearing limestone layers, resulting in steep-sided hills on which Permian limestone and shale layers are exposed.

Konza Prairie upland vegetation is dominated by the native grass species big bluestem (*Andropogon gerardii* Vitman), little bluestem (*Andropogon scoparius* Michx.), and Indian grass (*Sorghastrum nutans* (L.)). Along the longitudinal stream gradient there are three relatively distinct zones of riparian vegetation: grasses dominate headwater ("prairie") channels (stream orders 1–2), followed by a prairie/shrub zone (orders 3–4) of riparian grasses, shrubs (smooth sumac, *Rhus glabra* L., and buckbrush, *Symphoricarpos orbiculatus* Moench.), and small trees (American elm, *Ulmus americana* L., and honey locust, *Gleditsia triacanthos* L.), and a gallery forest (orders 4–5) dominated by bur oak (*Quercus macrocarpa*) Michx.), chinquapin oak (*Q. muehlenbergii* Engelm.), hackberry (*Celtis occidentalis* L.), green ash (*Fraxinus pennsylvanica* Marsh.), and American elm.

A 5.4-km reach of the South Branch of Kings Creek (Fig. 1) was studied from October 1980 to September 1981; physical characteristics of this catchment are presented in Table 1. The majority (52%) of total channel length is in the prairie riparian zone, but because channels in this zone are narrow, they comprise only 20% of the total channel area. The South Branch catchment has not been grazed by cattle since 1977, and most of it has not been burned since at least 1968.

Flow in South Branch is spatially and temporally variable. Headwater channels are ephemeral (carry flow only in direct response to precipitation), and the majority of the remaining channels are intermittent, flowing for 2–10 mo each year. Perennial pools and flowing reaches occur near springs, with intermittent channels occurring both upstream and downstream. Cessation of flow in late summer is expected for the majority of the Kings Creek drainage in most years, with flow resuming at some time between September and May after groundwater tables have recharged.

A U.S. Geological Survey (USGS) gage station (part of the USGS Hydrologic Bench-Mark Network) is located approximately 300 m downstream from the confluence of the north and south branches (Fig. 1). Water year 1980, which preceded this study, included a 4-mo flow period with only one substantial period of high flow in late March (1980A, Fig. 2). Although mean daily discharge on this date was high, the instantaneous peak (which describes erosional events better than mean daily discharge) was small compared with the peak in water year 1981 (1981C, Fig. 2). A long dry period preceded resumption of flow in May 1981.

Methods

Import

Direct litterfall was collected in plastic utility tubs (approximately 0.17 m^2 in area). Collectors were placed on level terrain adjacent to the stream channel. Small holes (approximately 2 mm in diameter) in the bottom of the tubs allowed precipitation to drain. Fifty litterfall collectors were placed at randomly selected intervals along the 5.4-km study channel of the South Branch drainage.

Lateral collectors were constructed of 6-mm-mesh hardware cloth attached to three sides of a steel frame (47 cm wide × 30 cm high × 35 cm deep); galvanized sheet metal covered the top and bottom. A canvas flap hung on the back side minimized sample loss due to winds from the direction of the channel. Twenty lateral collectors were placed at randomly selected locations along the study channel as close as possible to, and facing away from, the channel. Choice of left or right bank was also made randomly.

All collectors were in place on 1 October 1980. Collections were made biweekly through mid-December and then approximately monthly until mid-September 1981; a final 2-wk interval completed the 1-yr study period. Samples were placed in paper bags and transported to the laboratory, where they were sorted according to species or other litter category, oven-dried to constant weight at 50°C for 24–48 h, and weighed. Litter from each sorting category from each of the first seven collections was subsampled and combusted at 490°C for 6 h to

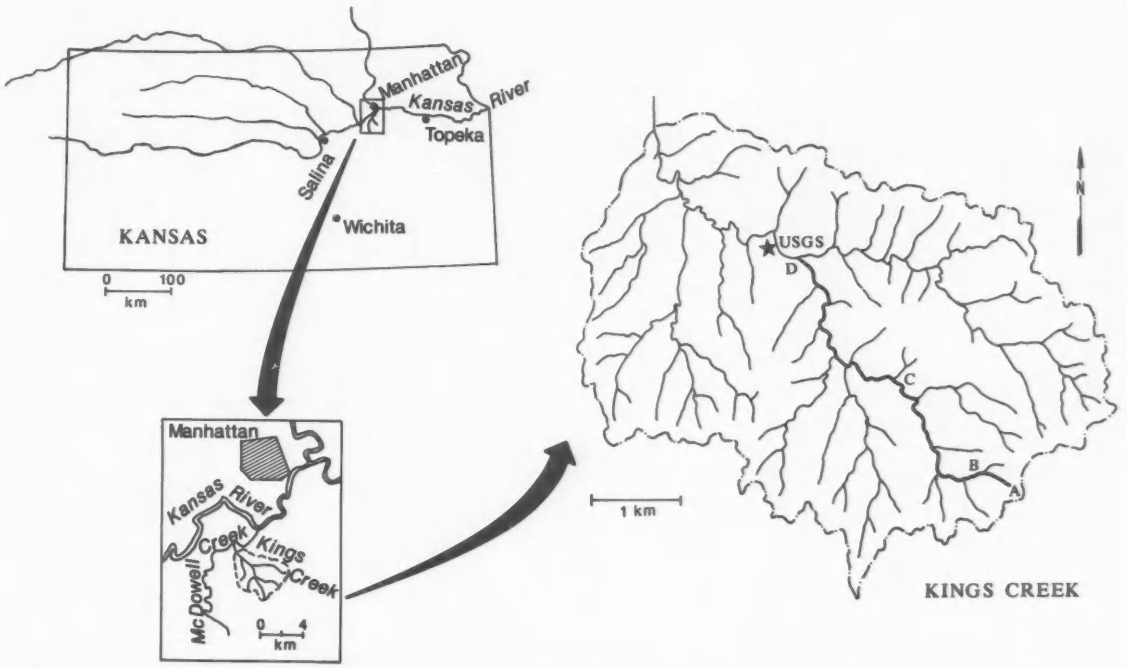

FIG. 1. Kings Creek drainage of Konza Prairie Research Natural Area, KS. The dark line indicates the 5.4-km study channel in the South Branch catchment. A–B = prairie zone; B–C = prairie/shrub zone; and C–D = gallery forest. Star symbol indicates location of U.S. Geological Survey gaging station.

determine percent organic matter. This procedure provided a species-specific correction yielding ash-free dry mass (AFDM).

Storage

A gasoline-powered vacuum collector ("D-Vac") was used to remove organic materials stored on the surface of dry channels. Deep storage was not sampled. Material was collected from the dry channel of South Branch in April 1981 ("spring" samples) before the beginning of flow and again in early September 1981 ("autumn" samples) after the channel had dried (Fig. 2) but before the peak period of litterfall. Thus, the spring sampling period collected organic matter that had accumulated since mid-July 1980, when the prior period of flow ended. Vacuuming allowed complete removal of all coarse materials from the dry channel, but fine particulate material, which can be a major component of benthic organic matter (Minshall et al. 1982, 1983), was not estimated. Furthermore, since storage estimates were made when the channel was dry, the standing crop of in-stream primary producers was zero. Algae and macrophytes contribute significantly to total storage in some streams (e.g. Naiman 1976; Fisher 1977).

Samples were collected from 25 transects at randomly selected locations along the 5.4-km study channel, perpendicular to the thalweg. Five transects were in the prairie riparian zone and 10 each in the prairie shrub and gallery forest zones. A 0.5-m-wide transect was established at each site and marked with flags; autumn transects were located 0.5 m upstream from the spring transects. The active channel was identified by a ridge at each edge and by abrupt changes in vegetation. Vacuumed samples of bank storage were collected from each side of the channel, extending the transect upslope to the top edge of the bank and to a height level with this on the opposite bank. High water marks of previous years were used to select the upper limit of each transect.

Organic matter was removed from portions of each of three sections (channel, left and right bank) by sampling from one or more 0.25-m^2 quadrats. In each transect, 52.1% (±2.6 SE) of the channel area and 55.0% (±2.8) of the bank area was sampled (total area sampled = 81.4 m^2). In the few cases where wood in the transect was greater than 10 cm in diameter, measurements were made for calculation of volume; mass was obtained by volume–mass regression.

Samples were dried and stored in paper bags. Material was washed and sorted into four litter types: leaves (excluding grass), grass tissues (leaves and stems), wood, and miscellaneous (unidentifiable plant pieces retained by a 1-mm-mesh screen), oven-dried at 50°C for 24–48 h, and weighed. Subsamples of each litter type were ashed in a muffle furnace (490°C, 6 h) and reweighed to determine percent ash.

Results

Hydrologic Conditions

During the study period, most intermittent channels of Kings Creek flowed only from May through August 1981. In lower reaches of South Branch, flow occurred from 18 May through

TABLE 1. Physical characteristics of South Branch catchment, Kings Creek, Konza Prairie Research Natural Area.

Watershed area (ha)	660
Maximum elevation (m)	430
Minimum elevation (m)	338
Main channel length (km)	5.38
Main channel gradient ($m \cdot km^{-1}$)	17.0
Prairie riparian zone	
Total channel length (km)	18.68
Mean width of active channel (m)	0.83
Bankfull width (m)	2.76
Total area of active channel (ha)	1.55
Total bank area (ha)	3.67
Prairie/shrub riparian zone	
Total channel length (km)	10.92
Mean width of active channel (m)	1.91
Bankfull width (m)	5.15
Total area of active channel (ha)	2.09
Total bank area (ha)	3.71
Gallery forest riparian zone	
Total channel length (km)	6.22
Mean width of active channel (m)	5.88
Bankfull width (m)	9.59
Total area of active channel (ha)	3.66
Total bank area (ha)	2.21

2 September; mean discharge at the USGS gage station during this period was 72.6 $L \cdot s^{-1}$. Highest flow during the study occurred on 27 June; peak discharge was 13 650 $L \cdot s^{-1}$. This storm flow caused considerable channel scour and rearrangement of channel substrates and woody debris. Although the period of record (7 yr) is short for flow frequency analysis (less than the 10-yr minimum recommended by the U.S. Water Resources Council 1981), estimates for the recurrence interval of this peak flow range from 2.4 yr (log-Pearson Type III) to 4.0 yr (Weibull plotting position $(n + 1)/m$, where n is the number of years of record and m is the rank of the annual peak discharge).

Imported CPOM

The storm flow of 27 June caused a loss of 12 direct samples and 6 lateral samples for the period 24–27 June; all lateral and two direct collectors were replaced on 29 June, with the remainder replaced on 13–15 August. A small number of samples were lost occasionally due to vertebrate influences (deer and wood rats).

In the prairie zone, direct import of CPOM was contributed mostly by grasses and herbaceous vegetation (Table 2); elm also contributed near the downstream boundary of this zone. Elm dominated direct inputs to the prairie/shrub zone, with honey locust, grass, oaks, smooth sumac, and buckbrush also common. Leaves of bur oak and hackberry, plus woody litter, comprised over 58% of the CPOM in gallery forest direct litterfall collectors, with chinquapin oak, green ash, and American elm also important contributing species.

The rate of direct litterfall was highest in the gallery forest; rates in the prairie/shrub zone were only 28% of those in the gallery forest. Direct litterfall in the upland zone may have been underestimated with this method because of the growth form of channel grasses; collectors were disruptive, since the grass stems were pushed away from the collector. However, prairie litterfall collections were made at several nearby sites on Konza Prairie during 10 June 1981 to 9 June 1982 using narrow troughs (5.6 cm wide × 1 m) inserted among grasses and removed and emptied periodically without disturbing the living or standing dead grasses (T. R. Seastedt, Kansas State University, unpubl. data). We estimate total annual litterfall (Table 2) of 122.8 g $AFDM \cdot m^{-2}$ from data on sites having vegetation similar to that of upland (prairie) South Branch stream channels.

As with direct litterfall, import from lateral movement in the prairie zone was mostly grass, with some herbaceous contributions (Table 2). Difference in species composition between direct and lateral collections for a given region is related to the random placement of the collectors and the spatial heterogeneity of the riparian vegetation. Material moving laterally into the stream channel in the prairie/shrub zone was mostly elm and grass, with ironweed, woody litter, buckbrush, and willow less common. In the gallery forest, leaves of oak, hackberry, and sycamore, as well as woody litter, were all well represented in lateral collections. Annual import rates from lateral movement were very low in the upland and prairie/shrub regions compared with the gallery forest.

Total annual import rates were obtained by adding direct litterfall to lateral movement rates (converted to an areal basis by multiplying the rate per metre of channel length by 2 and dividing by mean channel width). The highest rate was 482.2 $g \cdot m^{-2}$ in the gallery forest, while prairie/shrub (119.3) and prairie (135.6) zones were both much lower.

Lateral movement made up a larger percentage of total import in downstream reaches. In the prairie zone, lateral inputs were approximately 9% of the total; in prairie/shrub, 16%; and in gallery forest, 26%.

Storage

The quantity, composition, and dynamics of stored CPOM (>1 mm) varied among the three riparian zones (Fig. 3). Channels in the prairie and prairie/shrub zones had similar small amounts of CPOM in storage before the flow. Storage in the gallery forest channels was greater. Composition of stored CPOM in both channel and bank varied with changes in riparian vegetation; grass dominated channel storage in the prairie zone, while leaves and wood were more abundant in lower reaches. Bank storage was greatest in the gallery forest zone and least in the prairie/shrub zone before the flow period. Distribution of CPOM in the channel and on the bank was aggregated or patchy, i.e. the variances were high (Table 3). The distribution was most aggregated in downstream reaches, indicated by medians much lower than the means (Fig. 3).

The quantities of benthic CPOM in prairie/shrub and gallery forest zones were sharply lower after the flow period, but prior to leaf-fall. Storage in the channel in the prairie zone changed very little. Bank storage, on the other hand, declined somewhat in the prairie zone but increased in downstream reaches, suggesting that a net movement of materials from channel to bank occurred during storm flows.

Total CPOM storage was estimated by multiplying CPOM densities in sample transects by the estimated areas occupied by the channel and bank in each zone (Table 4). Net loss during the flow period represents fluvial export and breakdown to particles smaller than 1 mm, in excess of import. Approximately 29% of the annual import to the entire South Branch channel

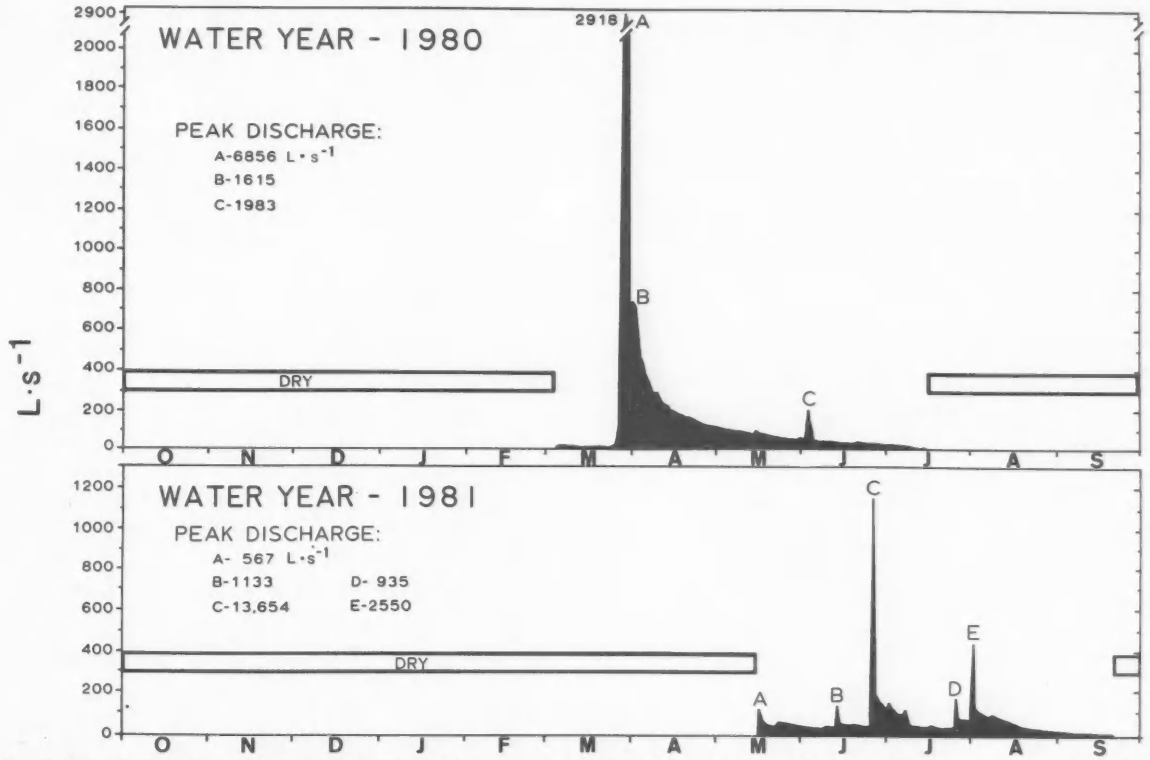

FIG. 2. Kings Creek hydrograph (mean daily discharge) for water years 1980 and 1981 (U.S. Geological Survey). Instantaneous peaks for major storm flows are also shown.

occurred during the 5 mo between the spring and autumn collections. Net loss of CPOM in the channel during the flow period was least in the prairie zone (14.7%). The increase in unidentifiable organic fragments (miscellaneous) in the prairie zone suggests that some of the CPOM present in the spring was broken down to smaller particles rather than exported. In addition, there was some contribution to the miscellaneous category from standing dead grasses remaining in the channel from the previous growing season. Proportionately more leaf material was lost from the channel than other types of organic matter, whereas net loss of grass was lowest. The loss of considerable amounts of wood from the channel probably occurred during the major storm flow on 27 June.

Increases in total storage of CPOM on the bank, except in the prairie zone, suggest that some of the materials entrained during high water stages were trapped in riparian vegetation downstream. More wood was collected in autumn than in spring; this difference cannot be accounted for entirely by lateral movement from the channel to the bank, since the increase on the bank exceeded the decrease in the channel. Wood densities were more variable than other litter categories. Slope movement or new deadfalls and windthrows may have increased the amount of wood on the bank.

Discussion

CPOM Import

The downstream sequence for total CPOM import was headwater prairie reaches < prairie/shrub < gallery forest; this longitudinal pattern of higher import in downstream reaches is opposite that expected for the generalized river continuum stream.

There are few studies with which to compare litterfall in the lower-order channels of the prairie zone of Kings Creek. In Deep Creek, Idaho, a small stream in the northern cool desert of the Great Basin, where the dominant streamside vegetation is sagebrush (*Artemisia tridentata* Nutt.) and grass, total allochthonous import ranged from 0.5 to 12 g AFDM·m^{-2}·yr^{-1}, assuming 5 kcal·g $AFDM^{-1}$ (Minshall 1978). Allochthonous inputs were approximately 240 g AFDM·$^{-2}$·yr^{-1} (1215 kcal·m^{-2}·yr^{-1}) to Rattlesnake Springs, Washington, a spring-fed stream surrounded by shrub-steppe desert dominated by sagebrush, with low shrubs and grasses dominating the streamside vegetation in the study reach (Cushing and Wolf 1982). The upland and prairie/shrub reaches of South Branch received amounts of CPOM intermediate between these two examples.

Total annual direct litterfall into the forested reaches of Kings Creek (357 g AFDM·m^{-2}; 402 g dry weight·m^{-2}) was similar to estimates for other cool temperate deciduous forests (Bray and Gorham 1964; Meentemeyer et al. 1982) but less than previously reported in another Konza Prairie gallery forest study (mean annual litterfall 694 g dry weight·m^{-2}; Killingbeck 1986). Killingbeck and Wali (1978) reported annual litterfall of 389 g dry weight·m^{-2} in a North Dakota gallery forest. Killingbeck (1984) measured 468 g·m^{-2} accumulation in Kings Creek during a time period representing more than 98% of total annual litterfall.

TABLE 2. Percent composition of CPOM import from direct litterfall and lateral movement in three riparian zones of South Branch of Kings Creek during 1 October 1980 to 30 September 1981. Species comprising greater than or equal to 1% in at least one riparian or CPOM category and total annual import per metre of stream channel (AFDM) are shown.

Species	Common name	Prairie		Prairie/shrub		Gallery forest	
		Direct	Lateral	Direct	Lateral	Direct	Lateral
Platanus occidentalis	Sycamore	0.0	0.0	0.0	0.0	0.7	9.3
Celtis occidentalis	Hackberry	0.0	0.0	1.5	1.7	14.5	13.4
Ulmus americana	American elm	3.6	0.0	26.4	35.1	3.3	4.2
Quercus macrocarpa	Bur oak	0.0	0.0	3.4	0.1	31.4	33.1
Quercus muehlenbergii	Chinquapin oak	0.0	0.0	5.6	0.0	6.7	9.3
Salix spp.	Willow	0.0	0.0	<0.1	2.3	0.8	0.1
Cercis canadensis	Redbud	0.0	0.0	<0.1	<0.1	1.7	0.2
Gleditsia triacanthos	Honey locust	0.0	0.0	12.3	0.6	2.2	0.5
Amorpha canescens	Lead plant	0.0	2.5	<0.1	0.0	0.0	0.0
Amorpha fruticosa	False indigo	0.0	7.0	0.0	<0.1	0.0	0.0
Cornus drummondii	Dogwood	0.0	0.0	3.0	0.6	2.3	0.4
Rhus glabra	Smooth sumac	0.0	0.0	8.1	0.0	<0.1	<0.1
Fraxinus pennsylvanica	Green ash	0.0	0.0	0.1	0.0	6.3	2.0
Symphoricarpos orbiculatus	Buckbrush	0.3	0.0	4.7	3.1	0.2	0.1
Artemisia ludoviciana	Sage	6.4	0.0	2.0	0.5	<0.1	<0.1
Vernonia baldwini	Ironweed	11.6	0.0	2.9	6.2	0.1	<0.1
Woody material		0.0	0.0	2.1	3.4	12.5	16.6
Leaf stems		1.0	0.4	4.1	0.6	3.0	0.8
Fruits and seeds		7.8	0.0	7.2	0.4	5.3	4.8
Grass		56.7	87.5	10.1	32.9	1.1	1.4
Other		12.7	2.1	6.5	11.6	7.9	3.4
Total		100.0	100.0	100.0	100.0	100.0	100.0
Total annual import:							
Direct ($g \cdot m^{-2}$)		122.8		100.3		356.7	
Lateral ($g \cdot m^{-1}$)			5.3		18.1		368.9

Riparian vegetation can be both a source of CPOM and a trap that restricts lateral transport of CPOM to the channel. In the Bere Heath area of the Bere Stream, England, a tall wet meadow community adjacent to the stream inhibited wind transport of leaves (Dawson 1976). In the present study, retention of CPOM on land was highest in grassland reaches and decreased downstream; the dense growth of grasses along the channel in the upland zone, and riparian shrubs and herbaceous vegetation in the prairie/shrub reaches, apparently trapped CPOM and prevented it from moving laterally. This is opposite the longitudinal pattern in a Quebec stream system, where lateral inputs declined from nearly one half of the total in a first-order stream to only 7–10% in fifth- to sixth-order reaches (Conners and Naiman 1984). In Snively Springs, a cool-desert stream in Washington, lateral inputs also were proportionately highest (70%) in arid, open headwaters where riparian vegetation was sparse and less (about 50%) downstream where riparian vegetation was denser and/or the stream was more protected from wind (Cushing 1988). Stream width (Conners and Naiman 1984) and bank slope (Fisher 1977) have been suggested as controlling factors in the proportional contribution of lateral inputs. However, in the present study, the reversed longitudinal pattern suggests that growth form and density of riparian vegetation are important contributing factors.

CPOM Storage

In contrast with CPOM import, which had a longitudinal pattern reversed from the generalized river continuum stream, benthic storage of CPOM had a variable longitudinal pattern. Following a period of accumulation in a dry channel, the longitudinal storage pattern was consistent with that of riparian import, i.e. higher quantity in downstream reaches than in headwaters. The pattern was reversed after cessation of subsequent flow, i.e. higher storage in headwater reaches than downstream reaches; thus, the autumn observations followed the generalized pattern of the river continuum concept. Composition of stored CPOM corresponded to the species of riparian vegetation in each zone at both sampling times, demonstrating a clear linkage between riparian and channel organic matter.

According to the "sliding scale" adjustment of river continuum patterns, relatively high quantities of import and storage should be observed in headwaters even in streams that enter the continuum at different points (Minshall et al. 1985). This storage pattern was observed in South Branch only after the flow period due to a combination of stabilizing influences in headwater channels (e.g. retention in grassy channels) and destabilizing influences (flooding) that caused greater losses downstream. Further, the longitudinal pattern of CPOM storage in this stream is so strongly influenced by stochastic hydrologic processes (timing and duration of annual flow period; frequency, magnitude, and timing of floods) that biological communities may be unable to adjust to the poorly predictable CPOM resource. Therefore, functional group composition of invertebrates also may not follow typical river continuum patterns. Hence, the typically low densities of shredders in Kings Creek (Smith 1986), even in perennial reaches, may be due to the variable availability of CPOM. Winterbourn et al. (1981) reached similar conclusions concerning shredders in New Zealand streams.

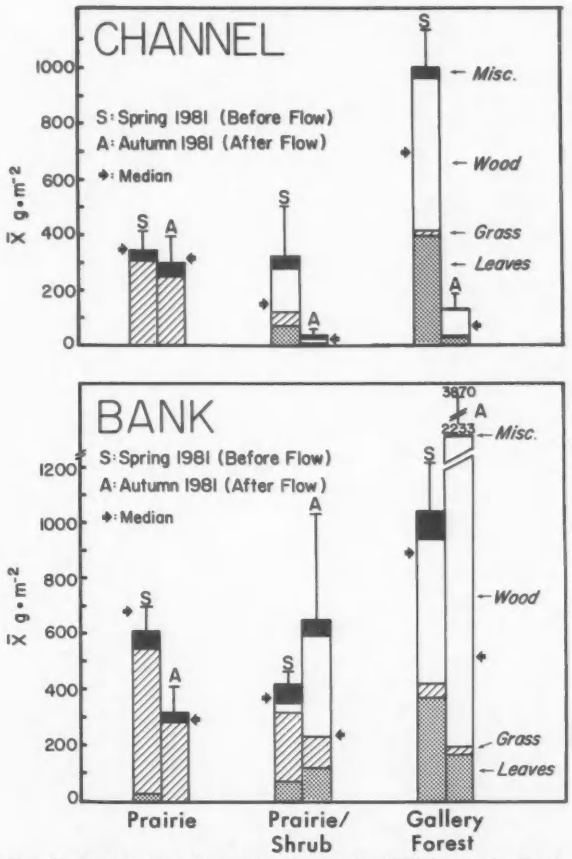

FIG. 3. Benthic organic matter storage (g AFDM·m^{-2}) in South Branch. Mean total storage (+1 SE) is shown for spring and autumn 1981 sampling periods in channel and bank areas of three riparian zones: prairie (0–0.5 km from the channel origin), prairie/shrub (0.5–2.8 km), and gallery forest (2.8–5.4 km); median values for totals are shown by bold arrows. Composition of organic matter by litter category is represented by shading.

Channel/Bank Comparisons of CPOM Storage

CPOM stored on the bank was both a source for the channel and, during storm flows, a trap for material entrained from the channel. More CPOM was stored on stream banks than in the channel. There were net losses of nonwoody CPOM from banks in all reaches during the flow period, but there were net gains of woody material to the banks in the prairie/shrub and gallery forest reaches. Thus, much of the woody material entrained in storm flows did not leave the watershed, but was moved onto the stream banks. The net change of CPOM stored in the channel between spring and autumn sampling periods represented export, decomposition, and lateral movement to the bank in excess of import of CPOM.

Comparisons of CPOM Storage with Other Streams

Storage of CPOM in gallery forest channels of the South Branch of Kings Creek was within the wide range of values reported in studies of streams with forested headwaters (Table 5). Only the prairie reach of South Branch, and Oregon streams (with wood included), did not show the large within-year variation common at other sites where more than one estimate was made for a given year. Total storage in the prairie reach was low compared with most other first- and second-order streams, but nonwoody material was not exceptionally low.

Wood is sparse in channels of South Branch compared with other streams in North America (cf. Wallace and Benke 1984; Harmon et al. 1986). The greatest mass of wood in the channel was only 0.56 kg·m^{-2} in the gallery forest channel in the spring (Table 2), a value lower than for any stream reported by Wallace and Benke (1984), with the exception of a southern Appalachian stream that had been clearcut with all slash burned 15 yr previously. The lowest value for wood mass in undisturbed channels reviewed by Harmon et al. (1986) was 0.7 kg·m^{-2} in Shinbuster Creek, California. The extreme rarity of bole wood (>10 cm in diameter) in South Branch (less than 0.02% of total channel or bank storage for either sampling period) contrasts sharply with Oregon streams draining old-growth Douglas fir catchments, where greater than 90% of stored CPOM was bole wood (Naiman and Sedell 1979). The low amount of wood in prairie or prairie/shrub riparian zones was expected. However, the potential for debris dam formation is present farther downstream, where the gallery forest extends 10–50 m on either side of the channel and mean annual litterfall rate exceeds that of most eastern deciduous and mixed deciduous forests (Killingbeck 1986). The potential for wood import is high in lower reaches of Kings Creek because of frequent high winds, ice storms, and high flows causing bank erosion. Nevertheless, large debris accumulations are typically rare in Kings Creek. Factors contributing to this rarity include (1) no contribution of wood from headwater reaches and (2) storm flows occurring with sufficient frequency and magnitude to scour debris dams and move large woody material either downstream or laterally to the upper bank.

CPOM Budget Considerations

Our study attempted to characterize two extreme conditions: a period following the autumn litterfall peak but before resumption of flow (and following 3 yr of below-average flow) and a collection after the summer flow period (which included one substantial stormflow). Our goal was not to estimate long-term mean conditions. Nevertheless, the reversal of longitudinal patterns of CPOM storage at these two extreme periods suggests that high variability would be expected if long-term measurements of this nature were continued. In this stream, the timing and duration of the annual flow period, and the timing and magnitude of stormflows, are highly variable. A better understanding of CPOM dynamics in this stream system requires long-term sampling with a frequency that is based on hydrologic as well as seasonal schedules.

To examine CPOM dynamics, and differences in storage both spatially and temporally, we compared the ratio of storage to annual import, on a total catchment basis, in the three riparian zones (Table 6). In spring, storage of nonwoody CPOM in prairie reaches was much greater than annual import, whereas in the other riparian zones, storage was more similar to the total annual import. There was little change in this ratio for the prairie zone from spring to autumn samples. Retention in these headwater channels was high because of ephemeral flow, low stream power, and efficient trapping of particulate matter by the grasses growing in the channel, despite the lack of woody debris which provides retention structure in other streams. Fur-

TABLE 3. Storage of CPOM (g AFDM·m^{-2}) in the channel and on the bank of South Branch during spring (April) and autumn (September) sampling periods; $\bar{x}$ = mean, SE = standard error, and n = number of samples.

	Channel						Bank					
	Prairie (n = 5)		Prairie/shrub (n = 10)		Gallery forest (n = 10)		Prairie (n = 4)		Prairie/shrub (n = 10)		Gallery forest (n = 10)	
	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
Spring												
Leaves	8	6	68	24	394	85	27	22	71	30	367	74
Grass	305	69	46	14	12	6	518	117	245	39	48	21
Wood	0	—	167	158	560	283	0	—	35	19	523	129
Misc.	29	12	40	13	33	8	61	9	62	12	98	15
Total	341	72	320	184	999	296	607	88	413	49	1037	186
Autumn												
Leaves	0	—	9	3	27	8	0	—	114	61	166	50
Grass	248	85	3	<1	3	1	283	62	96	12	31	15
Wood	0	—	15	14	97	38	0	—	369	327	2005	1606
Misc.	43	18	10	5	7	2	45	14	67	27	32	8
Total	291	100	36	17	134	47	328	71	646	410	2233	1636

TABLE 4. Total storage (kg) of benthic CPOM in the stream channel and adjacent bank in the three riparian zones of South Branch. Collections were made in spring (prior to onset of flow) and autumn (after flow ceased); net change represents losses or gains during the flow period.

	Channel				Bank			
	Prairie	Prairie/ shrub	Gallery forest	Total WS	Prairie	Prairie/ shrub	Gallery forest	Total WS
Leaves								
Spring	130	1530	14 650	16 310	990	2 650	8 130	11 770
Autumn	0	190	1 010	1 200	0	4 240	3 660	7 900
Net change	−130	−1340	−13 640	−15 110	−990	+1 590	−4 470	−3 870
% change	−100	−87.6	−93.1	−92.6	−100	+60.0	−55.0	−32.9
Grass								
Spring	5 120	1 030	440	6 590	18 990	9 100	1 070	29 160
Autumn	4 180	70	100	4 350	10 390	3 560	690	14 640
Net change	−940	−960	−340	−2 240	−8 600	−5 540	−380	−14 520
% change	−18.4	−93.2	−77.3	−34.0	−45.3	−60.9	−35.5	−49.8
Wood								
Spring	0	3 750	20 840	24 590	0	1 300	11 570	12 870
Autumn	0	330	3 620	3 950	0	13 680	44 330	58 010
Net change	0	−3 420	−17 220	−20 640	0	+12 380	+32 760	+45 140
% change	—	−91.2	−82.6	−83.9	—	+952.3	+283.1	+350.7
Misc.								
Spring	490	890	1 230	2 610	2 360	2 290	2 170	6 820
Autumn	720	230	250	1 200	1 650	2 490	700	4 840
Net change	+230	−660	−980	−1 410	−710	+200	−1 470	−1 980
% change	+46.9	−74.2	−79.7	−54.0	−30.1	+8.7	−67.7	−29.0
Total								
Spring	5 740	7 200	37 160	50 100	22 340	15 340	22 940	60 620
Autumn	4 900	820	4 980	10 700	12 040	23 970	49 380	85 390
Net change	−840	−6 380	−32 180	−39 400	−10 300	+8 630	+26 440	+24 770
% change	−14.6	−88.6	−86.6	−78.6	−46.1	+56.3	+115.3	+40.9

TABLE 5. Annual range of storage of CPOM (>1 mm; g AFDM·m^{-2}) in undisturbed streams. Where data are available, both total storage and total excluding wood are shown. In some cases, numbers reported here were derived from data given in the reference.

Stream	Order	Catchment area (ha)	Total/(excluding wood)	Reference (comments)
Catchment 54, NC	1	4	914/(186)	Wallace et al. 1982 (excluding large wood)
Grady Branch, NC	1	13	4470/(130–350)	Golladay et al., unpubl.
Hugh White Creek, NC	1–2	61	2960/(120–290)	
Bear Brook, NH	2	130	1190	Fisher and Likens 1973 (dry weight)
Covered Creek, AK	2	—	~40–120	Duncan and Brusven 1985
Little Beaver Creek, CO	3	4 600	~1–110	Short and Ward 1981
Fort River, MA	4	10 500	10–50	Fisher 1977
Devils Club Creek, OR	1	20	24150–26890/(400–3140)	Naiman and Sedell 1979
Mack Creek, OR	3	600	14290–15310/(40–1060)	
Lookout Creek, OR	5	6 050	5770–5950/(20–200)	
McKenzie River, OR	7	130 040	760–810/(10–60)	
Camp Creek, ID	2	75	30–182	Minshall et al. 1982 (wood >10 cm not sampled routinely but <5% except at Camp Creek)
Salmon River				
Smiley, ID	4	7 060	2–25	
Obsidian, ID	5	73 810	2–14	
Casino, ID	6	123 830	3–24	
Quebec				
First Choice Creek	1	25	6310/(316)	Naiman et al. 1987
Beaver Creek	2	183	4350/(125)	
Muskrat River	5	20 700	1350/(177)	
Matamek River	6	67 300	1270/(74)	
Moisie River	9	1 987 100	118/(40)	
South Branch, KS				
Prairie	1–2	20	291–341/(291–341)	This study
Prairie/Shrub	3–4	211	36–320/(22–153)	
Gallery Forest	4–5	660	134–999/(37–439)	

thermore, stems of senescent prairie grasses remain standing for at least a full year, assuring continuity of retention structure (Abrams et al. 1986). All other zones showed substantial decreases in this ratio from spring to autumn, losing about the equivalent of 1 yr of nonwoody CPOM import during the flow period; many years' equivalent of woody CPOM import were lost during the same period.

Both prairie/shrub and gallery forest zones were net exporters of CPOM, especially of wood, which had much less than the net annual supply remaining in the channel in autumn. This instability in storage contrasts, for example, with Oregon streams draining old-growth Douglas fir forests, where about 60 yr would be required to remove the storage of coarse wood (Cummins et al. 1983). Only the headwater channels had greater than a year's supply of CPOM remaining in autumn. Thus, this riparian zone may have particular importance to ecosystem processes downstream, as a stable organic resource and potential source of leachable dissolved organic carbon and other nutrients during periods of flow in the ephemeral headwater channels.

The channel and upper bank (floodplain) can be considered a single system (Table 6). Whereas net loss from the channel in the prairie zone was much less than annual import, losses from the bank exceeded losses from the channel so that, considered together, net loss exceeded annual import. Nonwoody CPOM also degraded (decreasing storage to import ratio from spring to autumn) in the other zones for both channel and channel plus bank. In contrast, ratios for wood decreased from spring to autumn in the channel, but increased in the channel plus bank, especially in the prairie/shrub zone. This difference suggests that much of the wood lost from the channel was retained on the bank.

Dynamics of CPOM storage depend on recent as well as historic hydrologic conditions. During the 3 previous yr without a major stormflow, CPOM may have accumulated in the system. The peak flow during the study period, with a recurrence interval of 2.4–4 yr, caused net losses that probably exceeded the long-term average.

Conclusions

Benthic organic matter is thought to be an integral component of the linkages between aquatic and riparian systems that

TABLE 6. Storage of CPOM and annual import of organic matter in three riparian zones of South Branch Kings Creek, and catchment totals. Values are kg totals for all channels in each zone as well as channel plus bank considered as a unit. Net change is calculated as the difference between autumn and spring storage and therefore reflects fluvial losses as well as breakdown to particles smaller than 1 mm.

	Prairie		Prairie/shrub		Gallery forest		Entire catchment	
	Nonwoody	Woody	Nonwoody	Woody	Nonwoody	Woody	Nonwoody	Woody
				Channel				
Storage (*S*)								
Spring	5 740	0	3 450	3 750	16 320	20 840	25 510	24 590
Autumn	4 900	0	490	330	1 360	3 620	6 740	3 960
Annual import (*I*)	2 100	0	2 430	60	15 260	2 390	19 780	2 440
Net change	840	0	2 960	3 420	15 000	17 220	18 770	20 630
Ratio *S*/*I*								
Spring	2.7	—	1.4	64.7	1.1	8.7	1.3	10.1
Autumn	2.3	—	0.2	5.8	0.1	1.5	0.3	1.6
				Channel + bank				
Storage (*S*)								
Spring	27 980	0	17 490	5 050	27 690	32 410	73 150	37 460
Autumn	16 930	0	10 770	14 010	6 400	47 950	34 110	61 960
Annual import (*I*)	6 410	0	5 690	120	18 330	2 610	30 420	2 730
Net change	11 040	0	6 720	−8 960	21 280	−15 540	39 040	−24 500
Ratio *S*/*I*								
Spring	4.4	—	3.1	42.1	1.5	12.4	2.4	13.7
Autumn	2.6	—	1.9	116.8	0.3	18.4	1.1	22.7

contributes predictability to longitudinal patterns in ecosystem characteristics, e.g. predictions of the river continuum concept. In the South Branch of Kings Creek, there is an expected change in the composition of import and storage of CPOM along a longitudinal gradient that corresponds to shifts in dominant riparian vegetation. However, benthic CPOM may not contribute consistently to the terrestrial/aquatic linkages in this prairie stream because of (1) a paucity of large CPOM sources (e.g. trees, shrubs) in the upper reaches and (2) a hydrologic regime that reduces the amount and the predictability of stored CPOM. Other sources of organic matter (e.g. dissolved organic matter in groundwater or leaching from terrestrial litter during storms, fine particulate organic matter from bank erosion, and instream primary production) are also subject to temporal variability associated with the hydrologic regime. Thus, biota of prairie streams must have opportunistic food gathering and reproductive strategies to take advantage of variable food resources in a flow environment that is itself very unpredictable.

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Effects of Low-Level Gas Supersaturation on Lake Trout (*Salvelinus namaycush*)

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Eggs, eleutheroembryos, and alevins of lake trout (*Salvelinus namaycush*) were reared in 1 of 10 levels of total gas pressure (TGP) ranging from 13 to 81 mm Hg (ΔP) above ambient barometric pressure. Rearing water was soft (30–40 mg $CaCO_3$/L, pH 6.8), temperature was 9.3°C, and the exogenous feeding portion of the test lasted 98 d. The supersaturation levels tested had no effect on the total survival of eggs, eleutheroembryos, or alevins, and condition factor and mean weight of fish in each treatment were statistically unchanged at the end of the study. Most of the fish that died during the feeding trial were small, had a below-average condition factor, and showed petechial hemorrhaging in the abdominal region. Length frequency distributions differed significantly among treatment groups after 56 d of feeding and remained different through 98 d. There was no difference in weight gain, condition factor, food conversion, and mortality, indicating that these measures are not useful for predicting or monitoring effects within the range of gas levels tested. The estimated incipient lowest level of effect of gas supersaturation remains unclear. A conservative recommendation of maximum supersaturation levels for small lake trout would be in the lower treatment levels used in this study.

Des oeufs, des éleuthéroembryons et des alevins de touladi (*Salvelinus namaycush*) ont été élevés dans des conditions correspondant à 10 régimes de pression de gaz totaux (PGT) allant de 13 à 81 mm de Hg (ΔP) au-dessus de la pression barométrique ambiante. L'eau utilisée était douce (30–40 mg $CaCO_3$/L, pH de 6,8), la température de 9,3°C et la période d'alimentation exogène de l'expérience a duré 98 d. Les niveaux de sursaturation utilisés n'ont pas eu d'effet sur la survie totale des oeufs, des éleuthércembryons et des alevins et le facteur de condition de même que le poids moyen des poissons ne présentaient, à la fin de l'étude, aucune différence statistique en fonction des régimes. La plupart des poissons qui sont morts au cours de l'essai d'alimentation étaient petits, présentaient un facteur de condition inférieur à la moyenne et souffraient d'hémorragies pétéchiales dans la région abdominale. Les distributions des fréquences de longueur de chacun des groupes d'essai différaient de façon significative après 56 d d'alimentation et ces écarts se sont maintenus jusqu'au jour 98. Le gain de poids, le facteur de condition, la conversion alimentaire et la mortalité ne présentaient aucun écart, ce qui montre que ces paramètres ne sont pas utiles à la prévision ou au contrôle des effets à l'intérieur de la gamme des pressions gazeuses utilisées. La valeur estimée du plus faible niveau de sursaturation gazeuse ayant des effets n'a pu être déterminée. Les concentrations gazeuses des gammes inférieures utilisées au cours de la présente étude semblent cependant correspondre au niveau de sursaturation sécuritaire dans le cas des petits touladis.

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Chronic or sublethal exposures of cultured fishes to supersaturated water supplies occur frequently in hatcheries supplied with groundwater sources. The pumping of water, or entrainment of air in pipes, may contribute to gas supersaturation and result in residual supersaturation even after the water is routinely degassed. Gas supersaturation has persisted as a problem in hatcheries, due primarily to the mechanics of handling large volumes of water.

In many studies, fish have been exposed to lethal supersaturation (Nebeker et al. 1978; Dawley et al. 1976; Bouck et al. 1976; Rucker and Kangas 1974). These studies of acute effects were not concerned with the subtle but debilitating changes that may occur in fish exposed to sublethal gas concentrations (see review by Weitkamp and Katz 1980). As a result, threshold gas levels for the safe and efficient culture of fishes have not been well defined for lake trout (*Salvelinus namaycush*).

Fidler (1984) described the biophysics of bubble formation in fish as a downloading of gas into tissue caused by changes in total gas pressure (TGP). He described two types of bubble formation: (1) progressive development in the circulatory system of fish, causing chronic gas bubble trauma (GBT), and (2)

simultaneous development of bubbles in all tissues, causing acute GBT. Alderdice and Jensen (1985b) deduced from the literature that the incipient lower level of chronic GBT begins at an increase in TGP (millimetres of mercury) above ambient pressure of 28–35 and that acute GBT begins a 60–76. The Environmental Protection Agency (Ebel et al. 1979) guideline for TGP (ΔP 76) is too high to protect hatchery fish from the chronic effects of supersaturation.

The objective of this study was to describe the effects of nonlethal gas supersaturation in lake trout, from eggs to juveniles of a total length of 6 cm, at gas levels of ΔP 10–80. The assignment of these concentrations, based on work by Alderdice and Jensen (1985b), created a study range from chronic to acute supersaturation and included their estimated incipient lower level of supersaturation (ΔP 30). Alderdice and Jensen (1985b) and Jensen et al. (1985) showed that the exposure time to 50% mortality (ET50) depends on TGP, fish size, the ratio of O_2 to N_2, and water depth. Therefore, these factors were controlled.

Eleutheroembryo, or free embryo (Balon 1975), describes the period from hatching to the beginning of exogenous feeding, and alevin (Balon 1980) describes the period from the beginning of exogenous feeding until lake trout reach a length of roughly 5.0 cm; thereafter the fish are considered juveniles until they become adults.

Materials and Methods

Water Supply System

The well water supply was divided into two separate water supplies, one of which was degassed and the other gasified (Fig. 1), so that treatment levels could be attained by mixing. All plumbing was polyvinyl chloride. Gas levels were maintained by mixing various ratios of the supersaturated and degassed water in 36-L tubs. Degassing was accomplished by passing water through pipe columns 152 cm × 30 cm diameter, filled with 3.8-cm packing rings (Glitsch Inc., Dallas TX 75266). The high-gas water supply bypassed the packed columns and was piped directly into a pressurized column (366 cm × 15 cm diameter). Water was supersaturated by pressurized air and bottled N_2 gas, injected at a rate of 4.3 m^3/d through two 5.0-cm^2 airstones (Aquatic Ecosytems, Apopka, FL) 0.3 m from the bottom of the column. Total air pressure

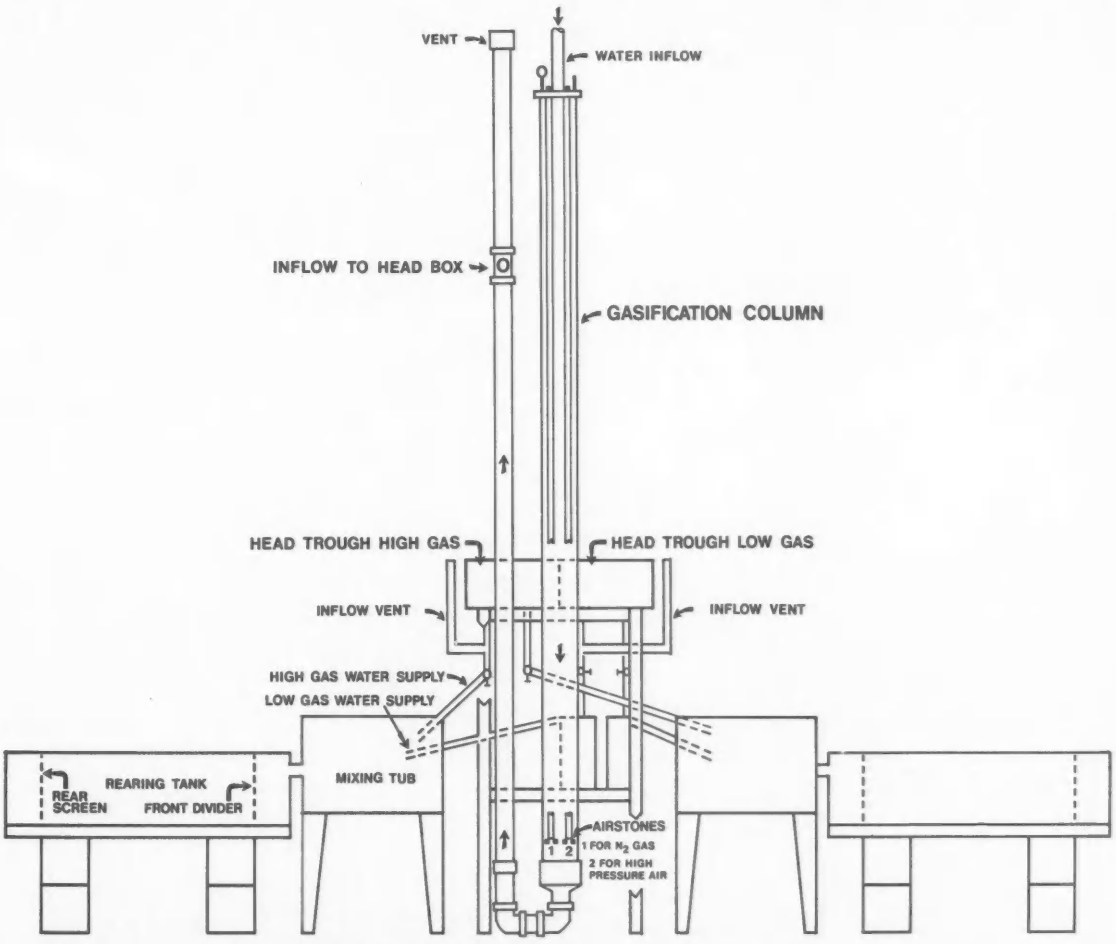

FIG. 1. Side view of fish culture system showing inflow system.

was maintained within the sealed column at 200 mm Hg. Water, introduced into the top of this column, flowed from a bottom outlet through a U-shaped, 9-cm-diameter pipe to a trough system (100 × 30 × 17 cm) that supplied the mixing tubs. Treatment gas levels were maintained by daily adjustment of flow ratios in mixing tubs. Flow to each rearing tank was 2 L/min. Mean water temperatures for the egg, eleutheroembryo, and exogenous feeding stages were 9.2, 9.5, and 9.0°C, respectively. Hardness and alkalinity ranged from 30 to 40 mg $CaCO_3$/L and from 24 to 48 mg $CaCO_3$/L, respectively; pH was 6.8. Half of each rearing tank was covered with plywood during the feeding phase of the study.

Rearing Tanks

High- and low-gas rearing waters were mixed in 36-L tubs and then distributed to rearing tanks (Fig. 1). Rearing tanks (122 × 61 cm) were glass and were divided along the longitudinal axis by a plywood partition. Actual rearing space in each aquarium section was 90 × 30 cm, with a water depth of 15 cm (40.6 L). Tanks were further divided in the front 15-cm space by a Plexiglas baffle (15 cm downstream from the inflow), with four holes 1 cm in diameter equally spaced at a height of 17 cm. Outflows were fitted with nylon mesh screen.

Gas Measurements

TGP was measured, as elevation of hydrostatic pressure above ambient saturation pressure (ΔP) (millimetres of mercury), with a gasometer (Bouck 1982). Other measured variables were barometric pressure, water temperature, and dissolved O_2, determined by the azide modification of the Winkler method (American Public Health Association et al. 1985). Supersaturation levels were established before the test by measuring in mixing tubs and outflow areas of rearing tanks. We first established that gas levels were the same at both locations and then measured one or two replicates of each treatment each week in mixing tubs so that fish would not be disturbed. Mean biomass per tank was only 77 g at the end of the experiment, and we did not monitor loss of dissolved O_2 during the study. The percentage TGP, partial pressures of O_2, N_2 + Ar, and the O_2:N_2 ratios were calculated by computer program (Dawson 1986) based on equations of Bouck (1982) and Colt (1983). In the discussion, estimates of ΔP from gas levels reported as percentages in the cited literature were based on a barometric pressure of 760 mm Hg. For example, a gas pressure of 110% TGP equals 10% supersaturation, or ΔP 76.

Gas levels for the 10 treatments ranged from ΔP 11 to 80 during the egg and yolk sac stages of the study. Gas levels were measured and reported as the positive pressure (ΔP) above the equilibrium gas pressure for environmental conditions of temperature and barometric pressure (D'Aoust et al. 1980; Colt 1980, 1983). Three replicate tanks were provided for each of 10 levels of gas supersaturation ranging from ΔP 11–13 to ΔP 80–81 (Table 1). At the end of the eleutheroembryo stage, gas levels were increased slightly, due to increased hatchery flows in packed to column degassers, to a ΔP range of 13–81.

Lake trout were reared at nearly constant gas pressures from egg fertilization to yolk absorption and for the feeding period of 98 d. The test began when water-hardened eggs (2 h post-fertilization) were placed into incubation trays. Three samples of 500 lake trout eggs (Seneca Lake, NY, strain) were weighed to determine the average. For each 10 treatment levels, three replicate groups of 500 eggs, estimated by weight at 46.2 g, were placed on wooden framed trays (19.3 × 24.5 cm) with nylon screen bottoms. Each tray was suspended 10 cm downstream from the tank inflow.

Handling and Calculation of Mortality

Beginning the 9th day after fertilization, dead eggs were removed and counted every 3–5 d throughout incubation. When 98% of all eggs were hatched, the numbers of eleutheroembryos were reduced to 100 per tray and the trays were inverted to release the fish into the tanks. Due to the low numbers of surviving embryos in some treatments (at ΔP 34, 41, 49, and 60), eyed eggs were added so that each tank contained 100 eleutheroembryos. All eggs that were added came from a tank of the same or next higher gas level. Eleven eggs per tank was the maximum number transferred (from ΔP 49 to 41). Ten each were transferred from ΔP 67 to 60 and all transfers at ΔP 34 and 41 came from their replicate treatment groups. Eggs that failed to hatch or that yielded deformed eleutheroembryos were not counted in the starting number of free embryos.

Feeding Test

At 40 d post-hatching, alevin numbers were reduced by random removal to 65 per tank, representing the minimum number fish available after length samples were removed. We weighed and measured 15 fish from pooled fish of each treatment. Total weight of alevins at the start was 7.2 g per tank. Sampling procedures were used to eliminate excess handling, and fish used for length determinations were not returned to treatment tanks.

Feeding, with a modified version of disk-type feeders (Falls 1980), was initially continuous over a 10-h feeding day (0700–1700). Food was SD-9, formulated by the U.S. Fish and Wild-

TABLE 1. Mean percent gas supersaturation levels (± SD) per treatment.

ΔP (mm Hg)	TGP (%)	O_2 (%)	N_2 (%)	Ratio O_2:N_2
13	101.9 ± 0.3	96.0 ± 3.6	103.5 ± 1.0	0.928
20	103.0 ± 0.3	98.0 ± 1.4	104.4 ± 0.5	0.938
29	104.0 ± 0.3	99.1 ± 1.4	105.3 ± 0.6	0.941
38	105.2 ± 0.4	99.0 ± 2.4	107.0 ± 0.6	0.926
45	106.2 ± 0.4	99.5 ± 1.6	108.0 ± 0.7	0.921
52	107.1 ± 0.3	101.8 ± 1.1	108.6 ± 0.4	0.938
57	107.8 ± 0.3	103.3 ± 1.2	109.1 ± 0.4	0.947
63	108.6 ± 0.2	102.6 ± 1.0	110.3 ± 0.2	0.931
72	109.8 ± 0.3	103.7 ± 1.1	111.6 ± 0.5	0.929
81	111.0 ± 0.3	103.8 ± 1.8	113.1 ± 0.5	0.917

life Service, Spearfish (North Dakota) Diet Development Center. We used starter diet for the first 17 d, No. 1 size crumbles for the next 42 d, and No. 2 crumbles thereafter. Fish were not fed on days they were sampled. The amount of food dispensed was based on a lake trout hatchery production feeding schedule (D. E. Ostergaard, Allegheny National Fish Hatchery, R.D. 1, Box 1050, Warren, PA 16365, pers. comm.). After 4 wk of feeding, the disk feeders were removed and the fish fed by hand five times daily over an 8-h period (0800–1600). After the first month of feeding, fish were weighed and counted without anesthesia every 2 wk. Individual length samples were taken, with anesthesia (MS-222) at each weigh period, except the next to last period. Daily photoperiod (overhead lighting) was 9 h light (0730–1630) : 15 h dark. Indirect sunlight from hatchhouse windows extended the photoperiod to natural day length, which gradually increased after the eggs hatched.

Statistical Analysis

The one-way analysis of variance (ANOVA) and the Kruskal–Wallis ranked test (Neter et al. 1985) were used for the statistical analysis of survival of eggs, eleutheroembryos, and alevins and for analysis of food conversion, weight per fish, and condition factor of alevins. The Kruskal–Wallis test and chi-square test were used to test for differences in fish length frequency distributions among treatments. Tukey's multiple comparison of means was used with analysis of variance. Chi-square percentiles were calculated from Thompson (1941). We rejected the null hypothesis whenever the level of significance was $P < 0.05$.

Results

Mean egg hatch per treatment was low and ranged from 16 to 32%, with the two highest hatching rates among eggs at ΔP 53 and 67 (Table 2). Eggs from the same parental stock, used for a hatchery production cycle, showed a similar or lower survival rate. Survival of eleutheroembryos and alevins were 79–89% and 74–82%, respectively (Table 2). ANOVA and the Kruskal–Wallis test showed no differences in mortality that could be attributed to the gas levels at any life stage tested. Variability within egg treatment groups was great (Table 2), probably due to differences in the quality of the eggs from first-spawning females or damage to adults incurred during transport to our facility a few months prior to spawning. Egg mortality rates varied between pre- and post-eyed stages. Variability (SD) within survival was different among treatments between the two stages (Table 2). Patterns of variability, however, do not show a relationship with increasing chronic gas levels. An undetermined number of unfertilized eggs was removed from each tray after eggs had fully eyed. Other egg deaths occurred throughout incubation, without treatment-related patterns. Survival of lake trout to swim-up was relatively unaffected by supersaturation up to ΔP 80, and survival rates varied little as gas pressures increased. Mortality did not increase when fish began to eat pelleted feed.

Nearly all fish that died at hatching had obvious anomalies. Mortality due to blue-sac disease was tracked throughout the free embryo stage. Blue-sac, a hydrocele condition of excess fluids and bluish color, was considered common in the *Salvelinus* by Wolf (1969), who attributed the disease to poor environmental conditions, possibly including collection of air bubbles on eggs. Blue-sac deaths accounted for only 1.0–2.3% loss, and ANOVA revealed no significant differences in incidence of blue-sac mortality. Among treatments, there were no significant differences in average weight/length (Table 3) or mortality of fish throughout the feeding study. Greater mortality occurred in the first 20 d in fish held at the three highest gas pressures, indicating that early mortality may depend on gas content if it exceeds ΔP 60. In all treatments, total mortalities increased from about 10% at day 40 to more than 20% by day 80 (Fig. 2).

Nonparametric statistics were used because fish length samples were not normally distributed, treatment level distributions did not adequately resemble negative binomial distributions (Elliott 1971), and log or square root transformations did not result in normality. Length data for each treatment were put into frequency distributions to show increased variance (Fig. 3) with time. The Kruskal–Wallis test, the most sensitive method used to analyze length frequency groups, showed that distributions differed only after 56, 70, and 98 d.

Changes in length frequency distributions were apparently related to cessation of feeding by individual fish. From days 42 to 56, frequency curves flattened (Fig. 3), indicating an increased number of small fish. At 70 d, peaks occurred at less than median value at ΔP 38, 63, and 81, indicating that a large proportion of fish were smaller than the median in these treatments. At 98 d (Fig. 3), the sample population peak shifted closer to the median value; distributions were similar, except that a large number of small fish remained at ΔP 81, apparently due to slow recovery or continuation of losses. The population

TABLE 2. Mean percent survival (± SD) for three replicates of lake trout eggs, eleutheroembryos, and juveniles reared at 10 supersaturation levels.

Gas level (ΔP)		Fertilization to:			
Egg phase	Hatch to termination	eye up	hatch	Hatch to swim-up	Growth study (98 d)
11	13	56 ± 4	23 ± 13	79 ± 6	75 ± 12
21	20	43 ± 28	19 ± 20	86 ± 5	76 ± 8
34	29	65 ± 5	25 ± 10	85 ± 7	76 ± 10
41	38	62 ± 4	22 ± 14	74 ± 7	76 ± 10
49	45	64 ± 4	16 ± 16	85 ± 4	82 ± 11
53	52	66 ± 3	32 ± 5	87 ± 7	80 ± 9
60	57	56 ± 24	17 ± 21	83 ± 2	80 ± 6
67	63	63 ± 1	31 ± 16	84 ± 7	74 ± 6
71	72	54 ± 22	23 ± 19	80 ± 5	78 ± 9
80	81	49 ± 22	21 ± 18	89 ± 2	75 ± 4

TABLE 3. Growth of lake trout at chronic gas levels: length[a] (mm) of fish per sampling period (±2 SD). Average length at the start of the test was 26.9 ± 1.0 mm, $n = 60$.

Gas level (ΔP)	Days of feeding				
	28 d	42 d	56 d	70 d	98 d
13	34.9 ± 5.0	38.8 ± 6.6	44.6 ± 11.0	45.2 ± 14.8	61.6 ± 18.6
20	34.6 ± 4.8	40.1 ± 4.6	41.9 ± 10.4	46.6 ± 18.8	63.2 ± 16.2
29	35.1 ± 4.8	39.3 ± 6.6	43.2 ± 13.0	49.5 ± 18.0	63.7 ± 20.6
38	35.2 ± 4.2	40.4 ± 4.6	43.4 ± 11.6	47.7 ± 17.6	65.2 ± 15.0
45	34.7 ± 5.6	39.0 ± 7.0	43.2 ± 10.4	46.7 ± 18.4	62.7 ± 19.0
52	35.1 ± 5.4	40.0 ± 7.2	44.5 ± 13.4	52.0 ± 13.0	63.1 ± 20.0
57	36.0 ± 5.4	39.6 ± 7.0	42.8 ± 9.6	46.6 ± 17.6	64.1 ± 19.2
63	35.0 ± 4.8	39.1 ± 7.0	42.9 ± 10.2	44.7 ± 15.4	63.3 ± 18.6
72	36.3 ± 4.4	41.2 ± 6.2	44.6 ± 11.6	48.3 ± 16.4	63.9 ± 17.8
81	35.5 ± 5.2	40.4 ± 6.8	45.0 ± 10.6	44.2 ± 14.4	60.7 ± 22.8

[a]For sample days 28–70, $n = 30$ per treatment; for sample day 98, $n = 90$ per treatment.

shift as seen at ΔP 98 was attributed to increased mortality of small fish after day 60.

Loss of small fish may have been due to either direct lethal effects of high gas pressure or to social factors involved in obtaining food. Live fish sampled from these tanks had petechial hemorrhages located on the abdomen. Direct effect of supersaturation was the most probable cause of death, since small fish were seen feeding; there was no evidence of pathogenic mortality, and petechial hemorrhages as described by Stroud et al. (1975) were visible.

Feed conversion (weight of food fed/fish weight gain) averaged 2.22 (1.97–2.47) for all treatments (Table 4). There were no significant differences among treatments (one-way ANOVA) for either feed conversion or condition factor.

Discussion

Eggs are resistant to excess gas pressures because they are themselves pressurized. Internal hydrostatic pressure within salmonid eggs raises the lower threshold level of gas supersaturation. Alderdice and Jensen (1985a) described an increase in pressure to 15 mm Hg within the chorion of eggs 2 h after fertilization. Pressures then increase to 50–90 mm Hg by hatching; thus the threshold of equilibrium gas pressure is effectively increased to as much as ΔP 90. Fidler (1984) also described an increased solubility of gases in egg yolk, which would moderate the effect of supersaturation on eggs. In other bioassays, Rucker and Kangas (1974) found no chinook salmon (*Oncorhynchus tshawytscha*) egg mortality effect at ΔP 213, and Nebeker et al. (1978) showed no egg mortality in steelhead trout (*Salmo gairdneri*) at gas pressures of ΔP 204. Because gas at low concentrations has little or no effect on eggs, in this study we would expect no cumulative effect of gas pressure up to ΔP 81 on embryos before they hatch. Movement of eggs or just hatched eleutheroembryos from ΔP 67 to 60 and from ΔP 49 to 41 probably had little effect on the outcome of these treatments. Test animals were transferred at a time of adjustment to ΔP while they were also very tolerant to changes in gas levels.

After eggs hatch, mortality and the presence of gas bubbles on the skin are typical signs of excess gas pressure in the water. In the present study, free embryos were allowed access to the bottom of the tank while they absorbed the yolk; they remained on the bottom of the tank, which lowered slightly ($-\Delta P$ 11 per treatment) the level of supersaturation. During most of the early stages, lake trout remained on the bottom of the culture tank. Special attention was given to mortality at the end of this stage because Wood (1968) and Nebeker et al. (1976) reported that mortalities occurred when the fish were ready to begin feeding. The rapid death of fish in those studies might indicate an acute mortality pattern. In our study, this pattern did not occur.

This study was unique in that fish were exposed to a wide range of sublethal gas pressures from time of fertilization through 98 d of feeding. In contrast, other studies of chronic effects have been limited to one life stage (Poston et al. 1973; Dawley and Ebel 1975; Colt et al. 1985). The prolonged exposure had negative effects on the lake trout once they were feeding, although the effects did not become evident until 56 d. The tolerance level of lake trout eleutheroembryos to supersaturation was greater than that of larval striped bass (*Morone saxatilis*), since no GBT was evident at ΔP 22 or more as in striped bass (Cornacchia and Colt 1984). Jensen et al. (1986) stated that ΔP 30 appears to be the lower limit of effect on salmonid juveniles after 50 d of exposure, but few data were available for their analysis. For lake trout, the long-term effects of supersaturation at ΔP 13–81, from the egg through early feeding stages, were expressed only in alevins after about 60 d of feeding, and only as variability in length distributions. We were unable to determine length distributions differences among treatment levels because the data could not be suitably transformed for use in ANOVA.

Chronic effects of supersaturation, in which there is progressive bubble growth in the circulatory path (Fidler 1984), may account for visible petechial hemorrhages, the lack of other visible signs of GBT, and the continuous mortality that occurred during this study. Fish had petechial hemorrhages in the abdominal region similar to those described by Meekin and Turner (1974) and Stroud et al. (1975) who noted that petechial hemorrhages are present under conditions of chronic gas supersaturation. Hemorrhages were present in 38% of the fish in Meekin and Turner's (1974) bioassays. Stroud et al. (1975) attributed hemorrhaging to an advanced stage of chronic GBT. External blisters on the skin were not visible on lake trout in this study. External bubble formation on fish is usually associated with acute gas levels (Weitkamp and Katz 1980). However, rapid changes in gas pressures could lead to bubble formation in susceptible fish (Alderdice and Jensen 1985b), and the lack of rapid fluctuations in gas pressures in this study may account for the absence of visible external bubbles, especially at ΔP 81.

Water depth and the small size of the fish may have together moderated the effects of the gas pressures. The fish usually

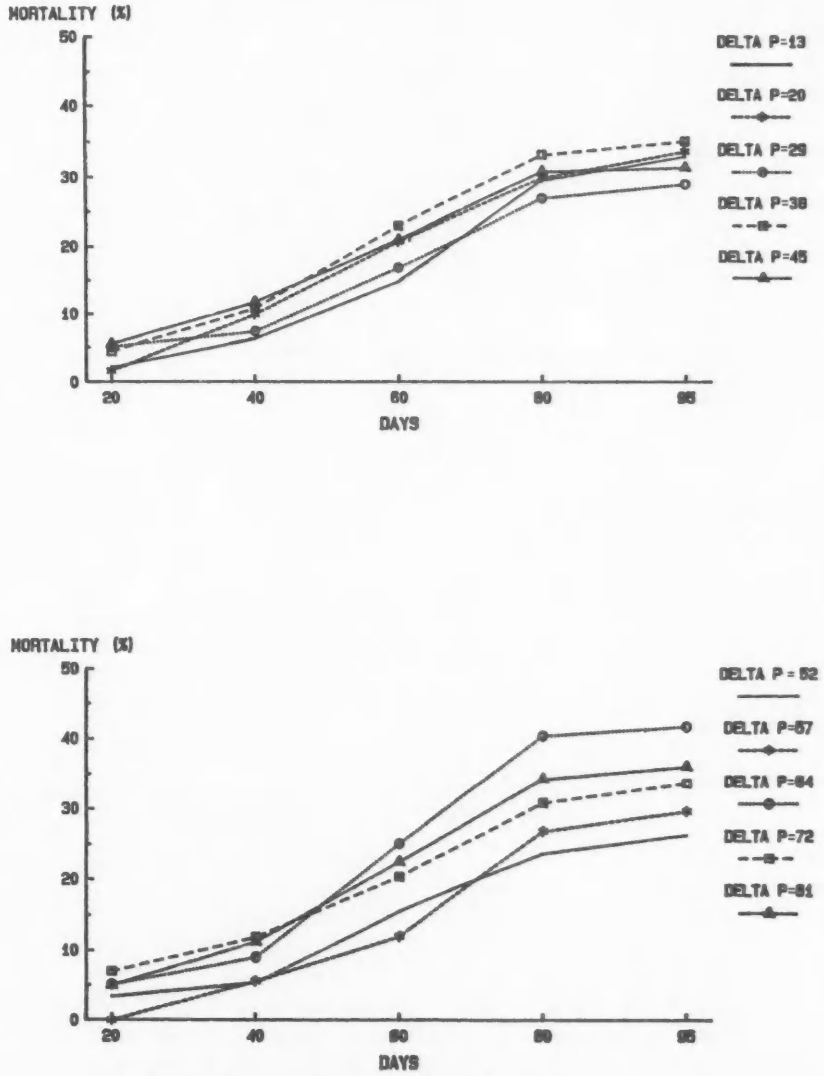

FIG. 2. Cumulative mortality of juvenile lake trout held at different concentrations of dissolved gases.

were near the bottom (15 cm depth), accounting for relief of about ΔP 11. Also, resistance to supersaturation tends to be greater in small fish than in larger juveniles (Jensen et al. 1985; Alderdice and Jensen 1985b). Gray et al. (1985) also showed size-related susceptibility in 96-h LC50 bioassays with post-larvae and fingerling sea bass (*Dicentrarchus labrax*) and striped mullet (*Mugil cephalus*).

Several investigators have reported reduced growth of juveniles or adults at elevated gas pressures. Poston et al. (1973) described a 15% growth reduction in rainbow trout and brown trout (*Salmo trutta*) held in water at 120% N_2 compared with those held at 107% N_2. The TGP for these studies was not given. Weights of yearling chinook salmon 115–120 mm long and steelhead trout 124–135 mm long were reduced at gas levels as low as ΔP 38 (Dawley and Ebel 1975), but lengths did not differ at the levels of supersaturation tested. The authors attributed the differential response in length and weight changes to the levels of supersaturation in the study. Signs of GBT and behavioral changes were not observed in these fish at gas levels below ΔP 76, but fish became lethargic and did not feed after 30 d at ΔP 114.

Daily food consumption of squawfish (*Ptychochelius oregonensis*) decreased by 84% as gas pressures increased from ΔP 0 to 152 (Bentley et al. 1976). The level of 50% reduction in feeding occurred at ΔP 114, indicating that reduction in feeding became rapid at gas levels above a threshold. Laboratory-reared lake trout fingerlings in a 29-d study showed 18% greater growth and 42% lower feed conversion at ΔP 7 than at ΔP 100 (Thorn et al. 1978); however, at ΔP 100, usually considered an acute gas pressure, growth reduction could have been much

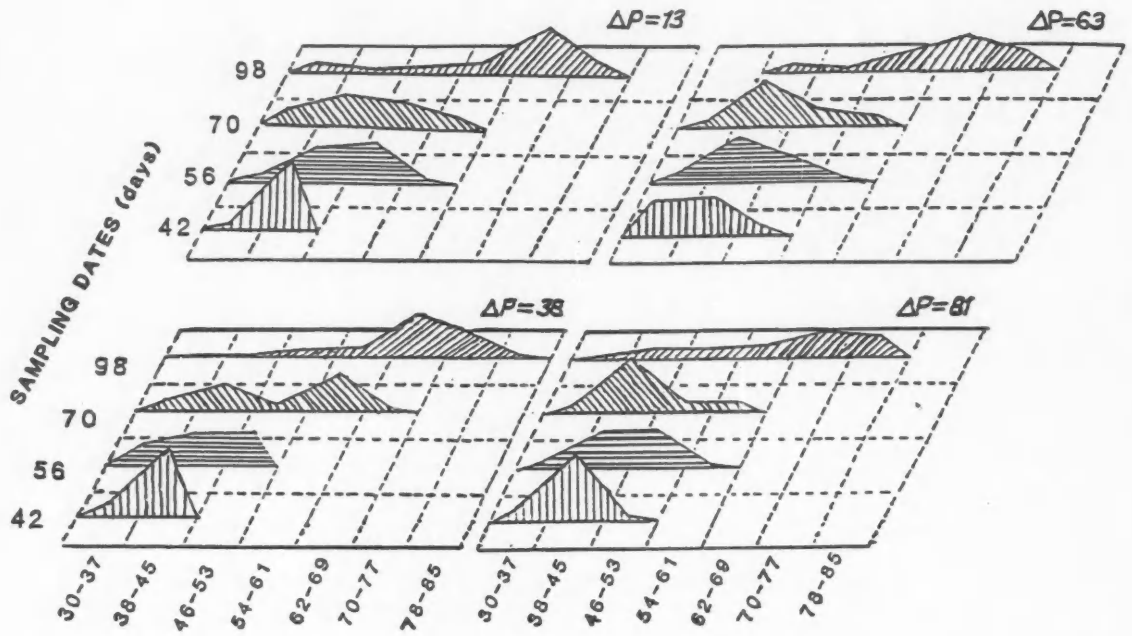

FIG. 3. Length frequency distribution of lake trout at 42, 56, 70, and 98 d.

TABLE 4. Feed conversion and condition factor of lake trout at each gas level.

Gas level (ΔP)	Feed conversion factor[a] (± SD)	Condition factor[b] ($\times 10^{-6}$)
13	2.38 ± 0.22	8.61
20	2.47 ± 0.70	7.29
29	1.97 ± 0.22	7.89
38	2.02 ± 0.07	7.13
45	2.26 ± 0.74	7.99
52	2.16 ± 0.28	7.76
57	2.23 ± 0.35	7.72
63	2.22 ± 0.50	7.50
72	2.10 ± 0.39	8.43
81	2.38 ± 0.30	8.08

[a]Feed conversion factor = weight of food/weight gain of fish.
[b]Condition factor = weight (g)/length (mm)^3.

greater. Changes occurring at low gas levels are similar among the various low levels, and differences may not be statistically significant. In the same study, results of 7-h bioassays at gas levels of less than ΔP 76–340 showed lake trout to be more tolerant than brook trout (*Salvelinus fontinalis*), brown trout, or rainbow trout to gas levels in excess of ΔP 230. Lake trout, however, were more susceptible to eye damage than the other salmonids.

Results from two chronic supersaturation studies showed that mean growth did not differ among channel catfish (*Ictalurus punctatus*) and chinook salmon. Colt et al. (1985) found no effect at ΔP 117 on mean growth in a 35-d feeding study with channel catfish. Wright and McLean (1985) also found no effect of ΔP 46 on chinook salmon in a 122-d study. Mortality was not significantly higher at ΔP 46 than at ΔP 8 with chinook salmon alevins (Wright and McLean 1985). At these sublethal gas pressures, Wright and McLean (1985) showed only slight differences in condition factor and mortality in summer chinook salmon over a 122-d rearing period, and most fish that died were fish that were much shorter (less than 2 SD) than the average. Also, no statistical differences were found for mean growth or feeding efficiency rates. This lack of significant differences may be misleading where sampling distributions deviate from normality as in length distributions.

High O_2 content in TGP may have a "sparing effect" on bubble formation (Alderdice and Jensen 1985b). If O_2 levels are low at a stable gas level, it is assumed that N_2 makes up the rest of the TGP, thereby increasing fish vulnerability to GBT. Although this phenomenon has been documented (Meekin and Turner 1974: Rucker and Kangas 1974; Rucker 1976; Nebeker et al. 1976, 1979; Machado 1984), there are no data to show whether there are threshold combinations of O_2:N_2 ratios that lead to bubble formation at low gas levels. Rapid changes in gas pressures, in either O_2 (Machado 1984) or TGP, as for instance when a fish rises from deep water, may predispose bubble formation. Fish tissue must adjust only once to a stable level of supersaturation. The rapid change hypothesis was supported by Machado (1984), who showed increased incidence of GBT when O_2 concentrations were doubled and N_2 was unchanged. Impacts of changes in levels of supersaturation were minimized in our study because both TGP and O_2:N_2 ratios were relatively unchanged during the tests. However, rapid changes in O_2 and N_2 are possible in culture facilities, especially in those that use vacuum or other degassing techniques, supplemental

(pure) O_2, or surface water with inherent diurnal fluctuations in dissolved gases and temperature.

The effects of cumulative growth of bubbles or their migration within the circulatory system over time are also unknown. Our mortality curves were similar for fish on all treatments (Fig. 2), suggesting that causes of death were similar at all treatment levels. Recommendation of maximum supersaturation for rearing of lake trout is based upon observed differences in length distribution (Fig. 3). To prevent bubble formation in lake trout eleutheroembryos and alevins, the maximum allowable level of supersaturation appears to be between ΔP 13 and 81. Results of this research do not indicate at which treatment levels fish lengths were statistically different; therefore, a maximum of ΔP 13 is a conservative recommendation.

It is difficult to determine the lowest level of supersaturation that will not adversely affect small lake trout. Growth and survival may not be affected in studies that last less than 6 mo. Measures of mean survival, mean growth, and mean condition factor appear to be inadequate to determine differences among fish at various sublethal levels of gas supersaturation. Identification of the level of gas supersaturation that results in a high percentage of fish with internal bubbles but without external signs would provide a useful indication of the incipient GBT level. Further research is needed to determine the actual gas level at which physiological damage occurs. Evaluation of immunosuppression or changes in blood ions or chemistry may also indicate the minimum point at which dissolved gas supersaturation affects lake trout.

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A Tag–Release/Recovery Method for Predicting the Effect of Changing the Catch of One Component of a Fishery Upon the Remaining Components[1]

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We describe (and illustrate, using data on southern bluefin tuna, *Thunnus maccoyii*) a tag–release/recovery experiment designed to predict the effect of changing the catch of one component of a fishery upon the remaining components for which fishing patterns and intensities are fixed. Formulae are developed for estimating the predicted changes and their standard errors. We also demonstrate how to determine the number of fish that should be tagged to achieve a desired accuracy of results from such an experiment. The proposed approach requires neither comprehensive knowledge of mechanisms governing the fish population and fishery being considered nor extensive historical catch and fishing effort data, but it will reflect all aspects of the dynamics of the system if the tag–release/recovery experiment is properly implemented. Consequently, it can be applied to many complex fisheries systems for which this knowledge is lacking. However, the proposed approach does not enable predictions to be made when the change in the catch significantly alters the number of recruits to the fishable stock, changes the fish behaviour, or changes the rates of natural mortality, migration, or growth.

Les auteurs décrivent (et illustrent à l'aide de données sur le thon rouge du sud, *Thunnus maccoyii*) une expérience d'étiquetage, de lâcher et de recapture conçue dans le but de prédire l'incidence qu'une variation des prises d'une composante de la pêche aura sur les autres composantes pour lesquelles les régimes et les niveaux de pêche sont fixes. Ils élaborent des formules d'estimation des variations prédites et leur écart type. Ils montrent aussi comment déterminer le nombre de poissons qui doivent être étiquetés pour obtenir la précision désirée des résultats d'une telle expérience. L'approche proposée ne nécessite aucune connaissance d'ensemble des mécanismes régissant la population de poissons ou la pêche étudiée ni un nombre important de données historiques sur les prises et l'effort; par contre, cette approche traduira tous les aspects de la dynamique du système si l'expérience d'étiquetage, de lâcher ou de recapture est adéquatement mise en œuvre. Par conséquent, elle peut être appliquée à de nombreux systèmes halieutiques complexes pour lesquels on manque de données. Toutefois, elle ne permet pas d'effectuer des prédictions quand la variation des prises modifie nettement le nombre de recrues au stock capturable ou amène une variation du comportement du poisson ou des taux de mortalité naturelle, de migration ou de croissance.

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Many fisheries, including those directed at single species, can be divided into easily distinguishable components. These components can be characterized by, for example, distinctive fishing methods or classes of fishing vessels, states or countries involved, and particular geographical areas or temporal patterns of operation (for examples of such fisheries, see Winters and Beckett 1978; Majkowski and Hampton 1983a; Moore and MacFarlane 1984). To what extent these components interact is of concern to fisheries scientists, managers, and fishermen. A knowledge of these interactions is essential for rational management of multicomponent fisheries and the resolution of frequent conflicts of interest among fishermen from different fishery components (see Alverson and Paulik 1973; Majkowski et al. 1981; Bronte and Johnson 1983; Kearney 1983). For example, it is important to be able to predict the effect that opening a new fishery component or closing an existing one has upon the catches of other existing components. Knowing this effect before the introduction of the proposed change to the fishery would allow fisheries scientists,

[1]An earlier version of this paper was presented during the Expert Consultation on Stock Assessment of Tunas in the Indian Ocean held in Colombo, Sri Lanka (Nov. 28 – Dec. 2, 1985) and its summary was included in the Collective Volume of Working Documents presented at the Consultation which was prepared by the Indo-Pacific Tuna Development Programme in 1986, p. 230–232.

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managers, and fishermen to assess whether this change is desirable or not. The Expert Consultation on Stock Assessment of Tunas in the Indian Ocean, held in Colombo, Sri Lanka (November 28 – December 2, 1985) recognized that the interactions among tuna fisheries in the Indian Ocean and other areas require urgent study (FAO 1985).

Fisheries systems, especially those having distinct components, are very complex. Hence, their mathematical or statistical models frequently have to be oversimplified and/or require extensive data sets. The interaction problem may be addressed by developing a mathematical mechanistic model of the fishery and population to describe the interactions among components of the fishery, estimating its input parameters and then assessing the effect of changes in the catch of one component on the basis of this model (for examples, see Lenarz and Zweifel 1979; Sibert 1984; Hampton and Majkowski 1986a). However, such an approach requires a comprehensive knowledge of the fish population and fishery (i.e. of the mechanisms governing the system and the characteristics of those mechanisms, including parameter values). In the case of Sibert's (1984) model the input parameters are estimated from tag–release/recovery data and commercial catch and fishing effort statistics whereas in the case of the remaining models, they are estimated from commercial catch and fishing effort statistics only.

The interaction problem may also be addressed by developing empirical statistical models or relationships, but again, such models or relationships require extensive empirical catch and fishing effort data on the effect of changing the current catch of a fishery component. Such approaches may be inconclusive and difficult to apply (e.g. Fonteneau 1986; Suzuki 1986). Experimental management regimes, as suggested by Sainsbury (1982), may be more conclusive, but they are also difficult to apply. Therefore, the development of alternative approaches for studying interactions should be continued.

In this paper we describe a new method for quantitatively predicting the effect of changing the catch of one component (A) of a fishery upon the catches of another component (B) and the entire fishery (E) where the fisheries components other than A are assumed to continue to operate with their established pattern and intensity of fishing. This experimental approach does not require the information necessary for applying the modelling and statistical approaches mentioned above, but its results closely account for all aspects of the dynamics of the system studied if the tag–release/recovery experiment is properly implemented and all assumptions are met. It can be applied, in particular, to complex fisheries directed at inhomogeneous populations or those, such as for tunas, for which migration is a major factor responsible for interactions. This approach may, therefore, be superior in many cases over the approaches outlined in the previous paragraphs, especially if the systems studied are very complex and extensive fisheries statistics are not available.

The general idea for such an experiment was originally suggested by Allen (1953) for predicting the effect of a small change in the minimum age at capture upon the yield-per-recruit, but the idea was not applied until its significant modification by Majkowski et al. (1984). Knight (1970) proposed an experiment based on the concept of Allen (1953) to predict the effect of a small increase in the number of fishing boats upon the total catch. To the best of our knowledge, the method of Knight (1970) has not been applied in practice.

By extending, in this paper, the approach of Majkowski et al. (1984), we significantly modify Knight's (1970) concept and demonstrate how it can be applied to the problem of quantitatively predicting the effect of changing the catch of one component of a fishery upon other components and the entire fishery. As with the maximum yield-per-recruit analyses of Ricker (1945), Allen (1953), Beverton and Holt (1957), and Sainsbury (1984), the experimental approaches of Knight (1970) and Majkowski et al. (1984) and some other fisheries calculations (for examples, see Majkowski and Hampton 1983a; Hampton and Majkowski 1986a), the approach presented here is applicable only if the level of recruitment to the fishable stock fish behaviour, and the rates of natural mortality, migration, and growth are largely independent of the magnitude of parental biomass, at least within the magnitude of fishing intensities applied. The case of fluctuations in recruitment to the fishable stock and other population attributes due to environmental conditions will be considered further in the paper.

This paper describes the design of the tag–release/recovery experiment and the method of analysis of the resulting data. In the design of this experiment, consideration is given to the numbers of fish that should be tagged in order to achieve a desired accuracy of results. The method of data analysis, including the estimation of accuracy of results, is illustrated using data on southern bluefin tuna (*Thunnus maccoyii*).

Formulation of the Problem

We will be predicting the effect of changing the catch of one component (A) of a fishery upon the catches of another component (B) and the entire fishery (E). The fishery components other than A are assumed to continue to operate with their established pattern (age selectivity and size selectivity, areas, seasons, etc.) and intensity of fishing. We also assume that the catches of components A and B are mutually exclusive. The increase, ΔY_A (or decrease, $-\Delta Y_A$), in the current weight of catch of fishery component A is defined as an attainable quota (i.e. one that is sufficiently small so that the population is not reduced to such low levels that the quota cannot be reached) that is to be taken in addition to (or foregone from) the current catch that is assumed to be also regulated by an attainable quota. We also assume that the age, spatial, temporal, and species distributions of these catch quotas are specified. The distributions of these attributes for the proposed quota change could be specified, for example, as being the same as for the current catch. If the quota of fishery component A is to be increased (or decreased), it is assumed that the catch of any subcomponent of A will not decrease (or increase, respectively). For example, if catches of component A are composed of 2- and 3-yr-old fish, it is not permitted to *decrease* the catch of 2-yr-old fish and to simultaneously *increase* the catch of 3-yr-old fish.

We assume that the current catch quota has been in effect for at least as many years as the natural life span of the species being considered. Similarly, we assume that the future quota of component A will be unchanged for at least the same period. If the current weight of the catch of fishery component A is to be reduced, we similarly assume that the fish representing this reduction have the same characteristics as a part of the current catch. The effect of changing the catch of component A upon the catches of component B and the entire fishery (E) will be expressed as changes in the weight of their catches after their stabilization. The condition of full stability is not necessary, but it is introduced for the sake of simplicity.

Tag–Release/Recovery Experiment

A sample of fish representing the potential change in the current catch of fishery component A should be tagged and released to the water. The age, spatial, temporal, and species distribution of the fish tagged should be the same as those for the considered change in the quota. Taggers and commercial fishermen from component A should ideally rerelease any tagged fish they recapture. The need for this rereleasing is established in the next section and Appendix A. No fishermen from the other components need rerelease tagged fish, but if they do, then data from the later recaptures of these rereleased fish should be excluded from the analyses outlined in the next section. The method of calculating the number of fish that should be tagged in order to estimate, with a specified accuracy, the change in the current catch derived by the entire fishery (E) or by its component B is outlined in Appendix B.

If a fisherman from component A catches a fish with an attached tag, but cannot rerelease it because of, for example, its poor condition resulting from catching, then a fish with the same characteristics as the recaptured fish should ideally be tagged and rereleased as a replacement. If this tagging and releasing replacement fish is not feasible, tag–release/recovery data derived from the experiment outlined can still be used, but additional information on the instantaneous rates of natural mortality and permanent emigration has to be obtained. Often very approximate estimates of these rates are sufficient for this purpose (see the following sections).

Reviews of the methods of tagging can be found in Begon (1979) and Jones (1979). The tagging method chosen should satisfy the following conditions: (a) catching fish and subsequently tagging them does not harm or kill them or change their behaviour; (b) the instantaneous rates of fishing and natural mortalities and migration are the same for tagged and untagged fish; (c) growth rates of tagged and untagged fish are the same; (d) the tags are not shed; and (e) tags are visible, with numbers that can be read on recapture.

The geographical position, date, tag numbers, and weight (and length and age if possible) of each fish should be recorded at the time of tagging. Similar information should be sought when a tagged fish is recaptured by a fisherman. This information can be collected and processed in the way described, for example, by Majkowski (1982a) and Majkowski and Morris (1986). In some cases where some data are missing or conditions (a) to (e) are not complied with, mathematical adjustments can be made in the way suggested by Majkowski et al. (1984).

Method of Data Analysis

Ideal Experiment

If the tagging experiment is both designed and executed ideally, the changes in the catch of fishery component B and of the entire fishery E (ΔY_B and ΔY_E, respectively), both of which result from the proposed change (ΔY_A) in the current catch of fishery component A, can be predicted from the tag–release/recovery experiment. The formulae quantifying these predictions are

$$(1)\quad \Delta Y_B = -\Delta Y_A R_B / S$$

$$(2)\quad \Delta Y_E = \Delta Y_A[1 - R_{E-A}/S]$$

where S is the biomass of the fish tagged, R_B is the biomass of all tagged fish that are recaptured by fishermen from fishery component B, and R_{E-A} is the biomass of tagged fish recaptured by fishermen from all fishery components other than A (i.e. from fishery component $E - A$).

The intuitive justification for equation (1) is obvious. Assume that all fish representing the entire potential change in the catch of fishery component A were tagged and released (i.e. $\Delta Y_A = S$ for an increase and $\Delta Y_A = -S$ for a decrease). In such a case the biomass of fish recaptured, with tags, by fishermen from fishery component B would be equal to the predicted change in the catch of fishery component B. Assuming that equation (1) is valid, the change in the entire catch (see equation (2)) can be treated as a sum of changes in the catches of fishery component A (i.e. ΔY_A) and of the entire fishery with the exclusion of fishery component A (i.e. $-\Delta Y_A R_{E-A}/S$). The second change can be calculated on the basis of equation (1) assuming that B is defined, in this case, as the entire fishery with the exclusion of A. The deterministic validity of equation (1) is established in Appendix A using an age-dependent model of the population.

Formulae for estimating standard errors of ΔY_B and ΔY_E are presented in Appendix B. They are derived assuming a binomial distribution for recaptures with probabilities determined by the deterministic model presented in Appendix A. In this model it is assumed that each fish represented in ΔY_A has an equal chance of being tagged. If this condition is not met (e.g. fish are only tagged from a limited number of schools) and there is substantial heterogeneity in capture probabilities, growth rates, migration, or age structure among the different population subgroups, then the use of the formulae given in Appendix B may result in biased estimates of the standard errors.

If Fish Cannot be Rereleased

If there are any fish that were tagged at age m and not rereleased by fishermen, as required in the ideal experiment, and no equivalent fish were tagged and released as replacements (see the Tag–Release/Recovery Experiment section), the values of R_B and R_{E-A} will be smaller than they would be for the ideal experiment. To compensate for this bias, the biomass, S, of fish tagged at age m should be reduced (this reduced biomass will be referred to as S') in the following way.

Initially, only the effect of changing the catch of fishery component A upon fishery component B will be considered. For each fish, i, that is tagged at age m and recaptured, but not rereleased as required, S should be reduced by $w_i(f_{A+B,i})^{-1}$, where w_i is the weight of the considered fish at tagging and $f_{A+B,i}$ is the probability of survival, up to the age of recapture, t_i, of the tagged fish in the absence of any fishing (see Majkowski et al. 1984). For example, if a tagged fish is recaptured on the day it is released, then $f_{A+B,i}$ is equal to 1 and this is equivalent to tagging one fish less. A fish permanently leaving the combined fishing grounds before reaching the age t_i is regarded by us as having not survived *in these* grounds. Hence,

$$f_{A+B,i} = \exp\{-\int_m^{t_i} X_{A+B}(t')\,dt'\}$$

where $X_{A+B}(t)$ is the sum of the instantaneous rates of natural mortality and permanent emigration from combined grounds of fisheries components A and B. The above formula for $f_{A+B,i}$ is established in Majkowski et al. (1984), but for the case where permanent emigration does not occur, i.e. for $X_{A+B}(t) = M(t)$, the instantaneous rate of natural mortality. If X_{A+B} is constant, i.e. $X_{A+B}(t) = X_{A+B}$, it follows from the above equation that $f_{A+B,i} = \exp\{-X_{A+B}(t_i - m)\}$. Similar definitions for $f_{E,i}(t)$ and

$X_E(t)$ can be introduced and corresponding equations formulated if the effect of changing the catch of fishery component A upon the entire fishery is considered.

If (i) there is no permanent emigration from fisheries components A and B and the entire fishery E, (ii) $M(t)$ is constant, i.e. $X_{A+B} = X_E = M(t) = M$, and (iii) certain experimental conditions are met, the value of M may be estimated by the method of Hearn et al. (1987) on the basis of the same tag–release/recovery experiment. That method requires very few assumptions concerning the instantaneous rate of fishing mortality. Therefore, if assumptions (i) to (iii) are met, ΔY_B and ΔY_E can be predicted from only one experiment, which does not require rereleasing of tagged fish.

If the conditions mentioned in the previous paragraph are not met, the values of $f_{A+B,i}$ and $f_{E,i}$ may need to be estimated externally to the tag–release/recovery experiment (see the Example section and Majkowski et al. 1984), which may sometimes be difficult. Therefore, in such cases, the tagging experiment should, if possible, be designed and executed in such a way as to eliminate the need to estimate $f_{A+B,i}$ and $f_{E,i}$. If this has not been done and the $f_{A+B,i}$ and $f_{E,i}$ values, estimated internally or externally to the tag–release/recovery experiment, are highly uncertain, the effect of this uncertainty upon the results of the analysis can be examined on the basis of sensitivity analysis (for methods, see reviews in Majkowski 1982b, 1982c; Majkowski and Hampton 1983b).

Ideally the age, spatial, temporal, and species distributions of tagged fish contributing to S and S' should be identical for equation (A10) of Appendix A to be satisfied. If the intensity of fishing is low, the departure from this condition is not likely to be significant. However, if the rate of recaptures by fishery component A is substantial, the condition may be significantly violated. In this case, errors in the estimates of ΔY_B and ΔY_E that are caused by not complying with the condition may be considerably reduced by (i) partitioning component A into k age-class-related subcomponents A_1, A_2, A_3,..... , A_k and then (ii) substituting S'_j for S_j (for the definitions of S'_j and S_j, see below) in the following equations (which are equivalents of equations (1) and (2)):

$$(3)\quad \Delta Y_B = -\sum_{j=1}^{k} \Delta Y_{A_j} R_{B,j}/S_j$$

$$(4)\quad \Delta Y_E = \Delta Y_A - \sum_{j=1}^{k} \Delta Y_{A_j} R_{E\text{-}A,j}/S_j$$

where ΔY_{A_j} is the proposed change in the catch of component A_j (by definition of changing the quota, ΔY_{A_j} is automatically specified), S_j (assumed to be greater than zero) is the biomass of tagged fish from components A_j, S'_j is the discounted value of S_j to allow for the nonrereleasing of tagged fish, and $R_{B,j}$ and $R_{E-A,j}$ are the biomasses of fish recaptured by fishermen from components B and $E - A$, respectively, that were initially tagged at ages corresponding to ΔY_{A_j}. These additional equations may be applied in the cases where the distribution of the fish tagged is not the same as that of the considered change in the catch quota of component A.

Environmental and Recruitment Variability

The experiment may be repeated for a number of years to assess the effect of stochastic environmental fluctuations upon the interactions among fisheries components. In this case the ΔY_B and ΔY_E values can be estimated for each experiment separately and standard statistical methods (e.g. an analysis of variance) can be applied to test if these values and their variances differ significantly among years. If differences are not statistically significant, it may be appropriate to pool all the data and analyse the pooled data by the methods described earlier. If the number of recaptures is small, this method should be used with caution unless the differences are negligible; there may be real differences that have not been detected. The standard error of the mean over years may be estimated by an analysis of variance method. In this application the analysis of variance is being used as a preliminary screening device and then as an estimation tool. Questions that might arise about the assumptions underlying an analysis of variance are not as important in this latter context as in a hypothesis testing application. As before, the interpretation of the significance levels obtained from analysis of variance tests should be made with caution.

Fluctuations in the level of recruitment to the fishable stock will also cause fluctuations in the catches of component B and the entire fishery E. However, these fluctuations will not effect ΔY_B and ΔY_E (defined as the changes due only to the alteration in the quota of component A) provided ΔY_A is attainable each year (which may be difficult if the fluctuations are large). This can be explained in the following way. If all the fish representing ΔY_A were tagged and released each year, then after stabilization the annual catch of tagged fish by fishery component B would be equal to the predicted decrease (or increase) in the catch of fishery component B.

Example

Background Information

Southern bluefin tuna is a highly migratory species. It spawns in the waters between northwestern Australia and Java (see Fig. 1) from September to March (Shingu 1978; Anonymous 1984). Juveniles migrate to the waters off the coasts of Western Australia (WA), South Australia (SA), New South Wales (NSW), and Victoria (Vic). The general direction of their movement within the 200-nautical-mile (370.4 km) Australian Fishing Zone is from west to east, although some fish move in the opposite direction (Murphy and Majkowski 1981). Also, many fish emigrate from the nearshore waters off the WA, SA, NSW, and Vic coasts to join oceanic communities in the Southern Hemisphere (Shingu 1978; Murphy and Majkowski 1981). At the age of 8 yr they begin to reproduce.

The schools of immature southern bluefin tuna within the Australian Fishing Zone support Australia's most economically valuable fin-fish fishery (see Fig. 1 and Majkowski et al. 1981; Hampton et al. 1984; Majkowski and Caton 1984; Majkowski 1985; Hampton and Majkowski 1986b). The fishing methods used are pole and line, purse seining, and, to a small extent, trolling. Most fish caught by Australian fishermen before 1980 were 2–5 yr old, but at present, older fish are also harvested by Australians.

Southern bluefin tuna leaving the Australian fishing grounds become subject to intensive Japanese longlining (see Fig. 1 and Shingu 1978) and, since 1980, much less intensive New Zealand trolling and handlining (see Gibson 1984). The age range of most fish caught by Japanese fishermen before 1980 was 4–15 yr, and this range has remained largely unchanged in recent years. The New Zealand fishery mainly operates upon fish of ages 7–15 yr. As this brief description suggests, the population and associated fisheries are a complex system.

From 1976 to 1983 the numbers of southern bluefin tuna caught annually by Australian fishermen have more than

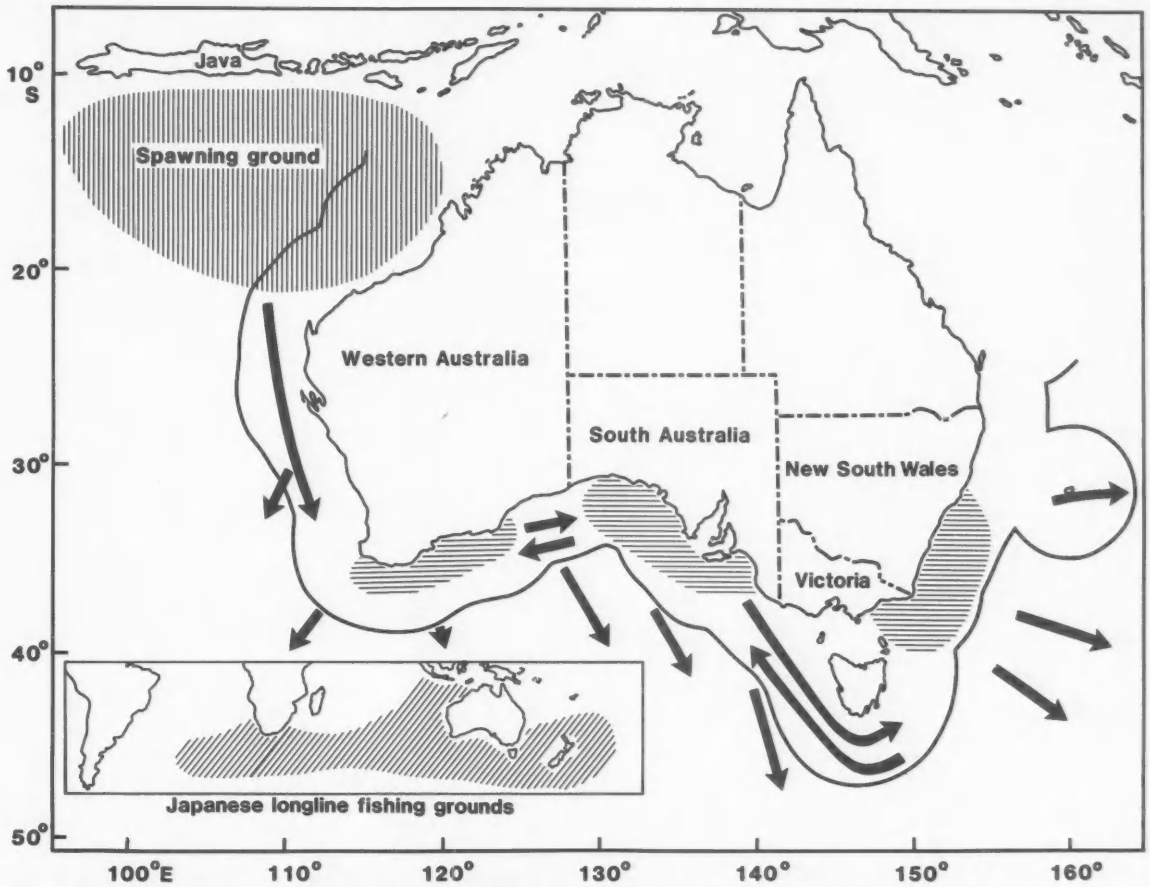

FIG. 1. Southern bluefin tuna spawning ground (vertically hatched area) and migration pattern off the Australian coast (arrows indicate the primary direction of migration; the boundary of the 200-nautical-mile Australian Fishing Zone is indicated by the solid line) and the areas of operation of the pre-1980 Australian fisheries (horizontally hatched areas) and the Japanese fishery (diagonally hatched area).

doubled. Most (80%) of this was due to the increase in the catches of the WA fishery during that period. As the tuna caught by WA fishermen are young (mainly 2 yr old), fishermen from other Australian states, who catch older tuna, but still immature (mostly younger than 6 yr old), and Japan are concerned about the effect that the WA fishery has upon their catches. Additionally, Japanese fishermen, who catch mostly mature tuna (up to 20 yr old), are concerned with the effect that the Australian catches of immature southern bluefin tuna have upon their catches. The methods presented here have been developed to address these problems.

Tagging Program

The Australian southern bluefin tuna tagging program was initiated in the late 1950s (for details, see Hynd 1969; Murphy and Majkowski 1981; Majkowski and Murphy 1983). Fish were caught (mainly by pole and line, but also by trolling), tagged, and released in the fishing grounds off the coasts of WA, SA, and NSW/Vic. Several thousand fish have been tagged in some years. Since the tagging program was not designed specifically to address the problem considered in this paper, the conditions specified in the Tag–Release/Recovery Experiment section were satisfied only in an approximate way; for example, fish recaptured by fishermen were very rarely rereleased.

Hynd (1963) mentioned that tagged fish under observation behave much like untagged fish. Additionally, growth, in length, of fish caught by the pole and line fishing method and tagged in the way described by Hynd and Lucas (1974) does not seem to be influenced by tagging after a few weeks (W. S. Hearn, unpubl. data), although some short-term effects are evident (Hampton 1986). Therefore, the effects of tagging are unlikely to be severe. Tagging is, therefore, assumed to result in little, if any, mortality (see condition (a)). Also for the same reason, tagged and untagged fish are assumed to be subject to the same rate of fishing and natural mortalities, migration, and growth (see conditions (b) and (c)). Nearly all fish were double-tagged with durable and easily noticeable (see condition (e)) standard tuna dart tags (Hynd and Lucas 1974).

The number of tagged fish recaptured but not reported is unknown. Nonreporting by Japanese fishermen may be quite significant. The number of reported recaptures (303) by these fishermen, out of 32 902 releases of fish tagged using the pole and line method in the late 1950s and 1960s, is small. Furthermore, reports of recaptures by Japanese fishermen have taken as long as 12 yr to reach tagging program organizers. It

seems likely, therefore, that many Japanese recaptures are not reported at all. The Australian and Japanese fisheries caught similar numbers of (untagged) southern bluefin tuna during this period. Although Australian fishermen reported 1620 recaptures, nonreporting by them may nevertheless be significant.

Data Analysis

To illustrate the proposed method of analysing tag–release/recovery data, the effects of potentially changing, in turn, the catch of the WA, SA, and NSW/Vic fishery components upon the SA and NSW/Vic components (but not the WA fishery, which was almost nonexistent during 1959–69) and the entire Australian fishery characterized by the fishing pattern and intensities of the 1960s are evaluated. As most of the releases of tagged fish took place during 1959–69 for which data appropriate to apply the method are now available, only the data obtained for these releases are analyzed. The releases are combined to make the results representative of the whole period. The age, spatial, and temporal distributions of the proposed catch changes are assumed to be the same as those of the fish tagged. The fish caught for tagging by trolling are excluded from the analysis because these fish may not satisfy condition (c) (see Majkowski 1982a).

In cases where the weight of a fish at release and/or recapture was not recorded, it is estimated on the basis of the weight–length relationship derived by Robins (1963) and the length at tagging and/or recapture, respectively. When the length at recapture is also unknown, it is estimated on the basis of the length at tagging, the period between release and recapture, and the length–age relationship (Kirkwood 1983). A statistical model developed by Kirkwood (1981) is applied to account for tag shedding (for further information on such an application and assumptions, see Majkowski 1982d; Hearn et al. 1987). The very few fish for which none of this information is available and could not be estimated are excluded from the analysis.

The entire Australian fishery consisted of the NSW/Vic and SA fisheries in the late 1950s and 1960s. Consequently, $X_{\text{NSW/Vic+SA}}$ can be assumed to be equal to X_{Aust}. In the absence of adequate information on the fishery components NSW/Vic + WA and SA + WA, we assume $X_{\text{NSW/Vic+WA}} = X_{\text{SA+WA}} = X_{\text{Aust}}$.

An estimate of the rate of exponential decay in recaptures by Australian fishermen was given by Murphy and Majkowski (1981) as $0.7 \cdot \text{yr}^{-1}$. This value is derived from the same tag–release/recovery data as those considered in this paper. The $0.7 \cdot \text{yr}^{-1}$ rate may be interpreted as the sum of the instantaneous rates of fishing and natural mortalities and permanent emigration. Therefore, as X_{Aust} does not include the instantaneous rate of fishing mortality, $0.7 \cdot \text{yr}^{-1}$ can be regarded as an upper limit of X_{Aust}.

On the other hand, it might be argued that there is little permanent emigration of southern bluefin tuna from the Australian fishing grounds until the age when they become subject to the Japanese longline fishery. In this circumstance, X_{Aust} would be equal to M, which was given by Hayasi et al. (1969) as $0.2 \cdot \text{yr}^{-1}$, but it might be as low as $0.1 \cdot \text{yr}^{-1}$ (see Majkowski and Hampton 1983a; Hampton and Majkowski 1986a). This implies that the instantaneous rate of fishing mortality for the Australian fishery is $0.5 \cdot \text{yr}^{-1}$ (or as much as $0.6 \cdot \text{yr}^{-1}$), which is generally thought to be too high because only 1620 tuna were recaptured by Australian fishermen, over a 2- to 3-yr period after tagging, from 32 902 that were tagged. This apparent anomaly may be due to an instantaneous rate of fishing mortality that decreases with age resulting in an exponential rate of decay in recaptures by Australian fishermen that is much larger than the sum of the instantaneous rates of natural and fishing mortalities.

Consequently, we assume $X_{\text{Aust}} = 0.4 \cdot \text{yr}^{-1}$, which is the mean of the two extreme estimates associated with the two different hypotheses on permanent emigration. To examine the sensitivity of results to changes in this assumption, we repeat the analysis using the extreme estimates of $X_{\text{Aust}} = 0.1$ and $0.7 \cdot \text{yr}^{-1}$.

Results

Changing the catch of any component of the Australian southern bluefin tuna fishery would have little effect upon the catches of the remaining Australian components characterized by the fishing patterns and intensities of the 1960s (Table 1). For each unit increase (or decrease) in the catch weight of either the WA, SA, or NSW/Vic fishery, the total Australian catch would increase (decrease) by 0.908, 0.989, and 0.974 unit, respectively. For each unit increase in the catch weight of the WA fishery, the SA and NSW/Vic catch weights would decrease by 0.061 and 0.031 unit, respectively. Similarly, for each unit increase (or decrease) in the catch weight of either the SA or NSW/Vic fishery, the catch weights of the NSW/Vic and SA fisheries would decrease (increase) by 0.011 and 0.026 unit, respectively.

The differences between the values of S and S' in Table 1 are small. This is because for the period under consideration, the Australian fishermen caught a low fraction (5%) of the tuna that were tagged. From the application of sensitivity analysis to this example, it is concluded that the predicted consequences that result from the changes in the SA and NSW/Vic fisheries, which are presented on Table 1, are insensitive to the $\pm 75\%$ changes in the assumed value of X ($0.4 \cdot \text{yr}^{-1}$). As a consequence of these $\pm 75\%$ changes, the $1 - R/S'$ values from Table 1 are altered by relative amounts in the range of $\pm 0.15\%$ and the R/S' values from Table 1 are altered by -3.4 to 5.6%. The predicted effects (given in Table 1) of changing the catches of the WA fishery are very insensitive ($\pm 0.02\%$) to the $\pm 75\%$ changes in the assumed value of X ($0.4 \cdot \text{yr}^{-1}$).

The results presented appear to indicate that there were only weak interactions among the Australian fisheries in the 1960s. This may be an artifact of a low reporting rate and/or of a substantially underestimated tag shedding rate. Alternatively, the interactions may have been weak at that time due to a low intensity of fishing, high emigration from the fishing grounds, and/or a very limited number of year-classes in the commercial catches.

The southern bluefin tuna population and the associated fisheries changes substantially during the 1970s and 1980s (see Majkowski et al. 1981; Hampton et al. 1984; Majkowski and Caton 1984; Majkowski 1985; Hampton and Majkowski 1986b). Hence, the values in Table 1 may not reflect the present situation which will be addressed on the basis of intensive tagging carried out by the CSIRO Division of Fisheries in 1983 and 1984 when sufficient data on recaptures are accumulated from this program. Also, the values in Table 1 may not reflect the true situation in the 1960s if nonreporting by Australian fishermen was significant or if recruitment to the Australian fishery, fish behaviour, or the rates of natural mortality, migration, or growth would have substantially altered due to proposed changes in the catches. As stated previously, the purpose

TABLE 1. Results of the empirical determination of the effect of changing the catches of the WA, SA, and NSW/Vic fisheries (see the first column) upon the entire Australian (Aust), SA, and NSW/Vic southern bluefin tuna fisheries (see the last three columns). S is the biomass of fish tagged and S' is the S-value discounted for nonrereleasing (see the Method of Data Analysis section). NA = not applicable.

Fishery changed	S (kg)	S' (kg)	Effect[a] of changing, by 1 unit, the weight of catch of the fishery listed in the first column upon catches of		
			Aust ($1\text{-}R_{AUST}/S'$)	SA ($-R_{SA}/S'$)	NSW/Vic ($-R_{NSW}/S'$)
WA	108 754	107 941	0.908 ± 0.004	−0.061 ± 0.004	−0.031 ± 0.002
SA	74 749	71 338	0.989 ± 0.002	NA	−0.011 ± 0.002
NSW/Vic	33 364	28 405	0.974 ± 0.004	−0.026 ± 0.004	NA

[a]Value following the ± sign is the standard error of the estimate.

of presenting this analysis for southern bluefin tuna is to illustrate the experimental approach proposed by us rather than to reach any conclusions regarding the southern bluefin tuna fishery.

Concluding Remarks

The experimental prediction of the effect of changing the catch of one component of a fishery upon the remaining components requires the implementation of an expensive and time-consuming tag–release/recovery experiment. However, the other approaches for examining interactions among fisheries also usually rely on tagging to determine input parameter values.

As demonstrated, the correct design and implementation of the specific tag–release/recovery experiment eliminates the need for information on the rates of mortality, migration, and growth. This may be the major advantage of this approach over the other methods for studying fisheries interactions. However, if it is not feasible to implement the ideally designed experiment, the approach still can be successfully applied to at least some systems, as demonstrated in the Example section.

The described experimental method automatically takes into account the age structure of fish caught by relevant fishery components. In fact, the primary reason for rereleasing tagged fish is to properly allow for the age structure of fish caught by the fishery component (A) of which the catch is to be directly changed. However, it is often difficult to rerelease tagged fish unharmed or to obtain accurate records of all necessary information. Therefore, alternative ways of designing the experiment and analysing the tag–release/recovery data are proposed for these cases.

The experiment proposed here, that addresses the fisheries interaction problem, has some similarities to the one that allows the estimation of the yield-per-recruit by the method developed by Majkowski et al. (1984). Consequently, it may be possible to select some data from the interaction experiment that satisfy the assumptions of the yield-per-recruit experiment. This is an advantage, if it can be implemented, because tag–release/recovery experiments are costly and time-consuming. In such a situation the extent of fisheries interactions can be predicted and the yield-per-recruit estimated from one experiment.

The main problem with the experimental approach to predicting interactions among fisheries (as with the experimental approach of Majkowski et al. (1984) to estimating the yield-per-recruit) is ensuring that experimental conditions (a) to (c) are met. However, the same problem may also arise with approaches that involve estimating mortality, growth, or migration rates from tag–release/recovery data.

Because the results of the experimental approach are derived from simple mathematical formulae, the approach can be easily understood by fisheries researchers, administrators, and fishermen. Hence, the implementation of the approach may gain their cooperation.

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Appendix A: A Demonstration of the Rationale for the Equation $\Delta Y_B = -\Delta Y_A R_B/S$

Let $P(t)$ be the number of fish at age t in a population that are vulnerable to the entire fishery E. This number is determined by the following differential equation:

$$\text{(A1)} \quad \frac{dP(t)}{dt} = -P(t)[F(t) + X_E(t)]$$

where $F(t)$ is the instantaneous rate of fishing mortality for fish at age t and $X_E(t)$ is the sum of the instantaneous rates of natural mortality and permanent emigration from the entire fishery E. If fish are recruited at age r, then for $r \leq t$, the following equation holds:

$$\text{(A2)} \quad P(t) = P(r) \exp\{-\int_r^t [F(t') + X_E(t')]\, dt'\}.$$

Denote the instantaneous rates of fishing mortality that are associated with fishery components A and B by $F_A(t)$ and $F_B(t)$, respectively. Let $P_A(m)dm$ be the number of fish at ages m to $m + dm$ that contribute to the potential change (ΔY_A) in the current catch of fishery component A. (It should be noted that a negative value of $P_A(m)$ corresponds to a decrease in the current catch of fishery component A.) Using this notation the potential change in the current catch weight of fishery component A can be expressed as follows:

$$\text{(A3)} \quad \Delta Y_A = \int_r^\infty P_A(m)\, w(m)\, dm$$

where $w(m)$ is the weight of fish at age m.

Initially assume that ΔY_A is an increase. The $P_A(m)dm$ fish at age m to $m + dm$ that are proposed to be caught by fishermen from component A, as a part of the additional quota ΔY_A, are currently subject to mortalities due to natural causes, fishing by the remaining fishery components, and permanent emigration from the entire fishery. Hence, the abundance ($P_A(m,t)dm$) of these fish, at some older age t, which would have been available to components other than A under the present fishing

regime (i.e. in the absence of the quota increase ΔY_A), is determined by the following equation similar to equation (A2):

$$(A4)\quad P_A(m,t)dm = P_A(m)\exp\{-\int_m^t [F_{E-A}(t') + X_E(t')]\,dt'\}\,dm$$

where $F_{E-A}(t)$ is the instantaneous rate of fishing mortality associated with the remaining fishery components.

Because fishery component B contributes to $F_{E-A}(t)$, the current catch of this component will decrease as a result of the additional $P_A(m)dm$ fish being caught by fishermen from component A. Accordingly, the incremental catch ($-d\Delta Y_B$) foregone in the current catch weight of component B (as a result of the increased catch $P_A(m)dm$ of component A) can be presented in the following form:

$$(A5)\quad -d\Delta Y_B = \int_m^\infty F_B(t')\,P_A(m,t')\,w(t')\,dt'\,dm$$

and then, by substituting for $P_A(m,t)$ using equation (A4), $d\Delta Y_B$ can be calculated as follows:

$$(A6)\quad d\Delta Y_B = -P_A(m)\int_m^\infty F_B(t')\,w(t') \times exp\{-\int_m^{t'} [F_{E-A}(t'') + X_E(t'')]\,dt''\}\,dt'\,dm.$$

It can similarly be shown that equation (A6) holds where $P_A(m)dm$ is negative. In this case the catch of component B increases by $d\Delta Y_B$. Accordingly, for any possible function $P_A(m)$, the entire change (ΔY_B) in the component B can be calculated from the following equation:

$$(A7)\quad \Delta Y_B = -\int_r^\infty P_A(m)\int_m^\infty F_B(t')\,w(t') \times \exp\{-\int_m^{t'} [F_{E-A}(t'') + X_E(t'')]\,dt''\}\,dt'\,dm.$$

Denote by $N(m)dm$ the number of fish at age m to $m + dm$ from the proposed additional catch of fishery component A which were tagged and released to the water. The biomass (S) of all tagged fish at the time of tagging can be expressed as

$$(A8)\quad S = \int_r^\infty N(m)\,w(m)\,dm.$$

Because all tagged fish that are recaptured by taggers or fishermen from component A are rereleased (see the Tag–Release/Recovery Experiment section), the instantaneous rate of fishing mortality operating upon tagged fish is equal to $F_{E-A}(t)$.

If the fish tagged and released are a part of the current commercial catch of component A and if these fish would be landed in the absence of tagging (i.e. the level of fishing effort is not increased because of tagging), the release of tagged fish can be treated as a decrease in the catch of this component, i.e. $N(m)$ can be substituted for $-P_A(m)$ in equations (A4) to (A7). The biomass (R_B) of tagged fish recaptured by fishermen from component B can be treated as an increase in the catch of this component, i.e. R_B can be substituted for ΔY_B in equations (A4) to (A7) in addition to the substitution of $-P_A(m)$ by $N(m)$. As a result of these substitutions, R_B can be calculated on the basis of the following equation:

$$(A9)\quad R_B = \int_r^\infty N(m)\int_m^\infty F_B(t')\,w(t') \times \exp\{-\int_m^{t'} [F_{E-A}(t'') + X_E(t'')]\,dt''\}\,dt'\,dm.$$

As a consequence of the assumption that the tagged fish are representive of the proposed change (ΔY_A) to the annual catch quota of fishery A,

$$(A10)\quad P_A(m)/N(m) = \Delta Y_A/S$$

if either $0 \leq P_A(m)$ for all m (i.e. when ΔY_A is positive) or $P_A(m) \leq 0$ for all m (i.e. when ΔY_A is negative). This last condition results from the constraint that no $N(m)$ values can be negative. Substituting $P_A(m)$ from equation (A10) into equation (A7) and substituting R_B by the right-hand side of equation (A9) results in

$$\Delta Y_B = -\Delta Y_A\int_r^\infty N(m)\int_m^\infty F_B(t')\,w(t') \times \exp\{-\int_m^{t'} [F_{E-A}(t'') + X_E(t'')]\,dt''\}\,dt'\,dm/S$$

$$= -\Delta Y_A R_B/S$$

as required.

In this proof it is implicitly assumed that the only variability in the fish being tagged is in their ages. However, the formula may similarly be shown to hold if there is variability in other characteristics of fish being tagged. Such characteristics may be size, future growth, age at recruitment, or temporal and geographical distributions.

Appendix B: Standard Errors of ΔY_B and ΔY_E

Because ΔY_A is constant, standard errors of ΔY_B and ΔY_E (i.e. $SE(\Delta Y_B)$ and $SE(\Delta Y_E)$) can be calculated on the basis of the following equations:

$$(B1)\quad SE(\Delta Y_B) = \Delta Y_A VAR(R_B/S)^{0.5}$$

$$(B2)\quad SE(\Delta Y_E) = \Delta Y_A VAR(R_{E-A}/S)^{0.5}$$

where $VAR(R_B/S)$ and $VAR(R_{E-A}/S)$ are the variances of R_B/S and R_{E-A}/S, respectively. The variance of the ratio of two random variables ($VAR(U/V)$) may be found from a well-known approximation based on Taylor's expansion:

$$(B3)\quad VAR(U/V) = \mu_V^{-2}VAR(U) + \mu_U^2\mu_V^{-4}VAR(V) - 2\mu_U\mu_V^{-3}COV(U,V)$$

where μ_U and μ_V are expected values of U and V, respectively, and $COV(U,V)$ is the covariance of U and V.

It may be deduced from Goodman's (1960) discussion of the merit of this approximation that if the terms in a product are symmetrically distributed, the approximation is generally very good. For quotients the quality of the approximation declines as the coefficients of variation of the component terms and the correlation between them increases. For coefficients of variation less than 0.2 for both terms, a correlation less than 0.3, and normally distributed data, the percentage error is less than 4% and can be ignored. These conditions are satisfied in all cases analysed in the Example section (asymptotic normality holds by the central limit theorem).

The variances of R_B/S and R_{E-A}/S can be estimated if values of μ_S, μ_{R_B}, $VAR(R_B)$, $VAR(S)$, $COV(R_B,S)$, $\mu_{R_{E-A}}$, $VAR(R_{E-A})$, and $COV(R_{E-A},S)$ are known or can be estimated. The method of estimating these quantities is presented below.

Denote the probabilities of recapture by fishermen from components B or E, excluding A, by p_B and p_{E-A}, respectively. The numbers of recaptures by these components (n_B and n_{E-A}, respectively) are assumed to follow the binomial distributions with parameters p_B or p_{E-A}. Using the methods presented by Majkowski et al. (1984), it can be demonstrated that the variances of R_B and R_{E-A} (i.e. $\sigma_{R_B}^2$ and $\sigma_{R_{E-A}}^2$, respectively) can be calculated as follows:

$$(B4)\quad \sigma_{R_B}^2 = Np_B[(1-p_B)\,\mu_B^2 + \sigma_B^2]$$

(B5) $\sigma^2_{\hat{R}_{E-A}} = Np_{E-A}[(1-p_{E-A})\,\mu^2_{\hat{E}-A} + \sigma^2_{\hat{E}-A}]$

where N is the number of fish tagged, i.e.

$$N = \int_r^\infty N(m)dm$$

where $N(m)$ is defined in Appendix A and

$$\mu_B = n_B^{-1} \sum_{i=1}^{n_B} w_{B,i}$$

$$\mu_{E-A} = n_{E-A}^{-1} \sum_{i=1}^{n_{E-A}} w_{E-A,i}$$

$$\sigma_B^2 = (n_B - 1)^{-1} \sum_{i=1}^{n_B} (w_{B,i} - \mu_B)^2$$

$$\sigma^2_{E-A} = (n_{E-A} - 1)^{-1} \sum_{i=1}^{n_{E-A}} (w_{E-A,i} - \mu_{E-A})^2$$

and $w_{B,i}$'s and $w_{E-A,i}$'s are the weights of fish recaptured by fishermen from components B and $E-A$, respectively. It can be similarly shown that the variance of S (i.e. σ_S^2) is specified by the equation

(B6) $\sigma_S^2 = (N-1)^{-1} \sum_{i=1}^{N} (w_{A,i} - \mu_A)^2$

where

$$\mu_A = N^{-1} \sum_{i=1}^{N} w_{A,i}$$

and $w_{A,i}$'s are the weights of the fish tagged.

To demonstrate the technique of estimating the covariances, $\mathrm{COV}(R_B,S)$ will be evaluated:

(B7) $\mathrm{COV}(R_B,S) = \mathrm{COV}_{n_B}\{E(R_B \mid n_B), N\mu_A\}$

$$+ E_{n_B}\{\mathrm{COV}(R_B,S \mid n_B)\}$$

$$= 0 + Np_B\,\sigma_B\,\sigma'_S\,\rho_{B,S}$$

where $\rho_{B,S}$ denotes the correlation between weights at tagging and recapturing and E (for equation (B7) only) denotes the expected value of the quantity following. The last correlation can be estimated using the following formula:

(B8) $\rho_{B,S} = [(n_B - 1)\,\sigma_B\sigma'_S]^{-1} \sum_{i=1}^{n_B} (w_{A,i}\,w_{B,i} - \mu'_A\,\mu_B).$

The $w_{A,i}$'s used here are the weights, at tagging, of fish that are subsequently recaptured (i.e. that correspond directly to the $w_{B,i}$'s) and μ'_A and σ'_S are estimated only from the tagged fish recaptured by fishermen from component B. From the calculations presented above, it follows that

(B9) $\mathrm{VAR}(R_B/S) \approx (N\,\mu_A^2)^{-1}\{p_B\,(1-p_B)\,\mu_B^2 + p_B\,\sigma_B^2$

$$+ p_B^2\,\mu_B^2\,\sigma_S^2/\mu_A^2 - 2p_B^2\mu_B\,\sigma_B\,\sigma'_S\,\rho_{B,S}/\mu_A\}$$

where p_B and p_{E-A} can be estimated as n_B/N and n_{E-A}/N, respectively (see Majkowski et al. 1984). $\mathrm{VAR}(R_{E-A}/S)$ can be calculated identically except subscripts B in equation (B9) have to be replaced with $E - A$ throughout.

It may be desirable to calculate the number of tagged fish, N, that results in a specific ratio (θ) of the standard error of ΔY_B (or ΔY_E) to ΔY_A. This quantity has the advantage of being dimensionless. A formula for N may be obtained by noting that $\mathrm{VAR}(R_B/S) = \theta^2$. Substituting this into equation (B9) and rearranging gives

(B10) $N \approx (\theta\mu_A)^{-2}\{p_B(1-p_B)\mu_B^2 + p_B\sigma_B^2 + p_B^2\mu_B^2\sigma_S^2/\mu_A^2$

$$- 2\,p_B^2\,\mu_B\,\sigma_B\sigma'_S\rho_{B,S}/\mu_A\}.$$

The number of fish to be tagged that results in the specified ratio of the standard error of ΔY_E to ΔY_A can be calculated identically, except that subscripts B in equation (B10) have to be replaced with $E - A$ throughout.

If such a calculation were to be performed to aid in the design of an experiment, it would be necessary to know the values of μ_A, μ_B, σ_S, $\sigma'_S\,\sigma_B$, μ_{E-A}, σ_{E-A}, p_B, p_{E-A}, $\rho_{B,S}$, and $\rho_{E-A,S}$. Usually the parameters are unknown at this stage and it would be necessary to take the maximum of the N's calculated from equation (B10) over a plausible range of values of the unknown parameters.

An Experimental Study of the Plankton Community Impacts of Two Omnivorous Filter-Feeding Cichlids, *Tilapia galilaea* and *Tilapia aurea*

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We conducted laboratory selective grazing experiments and outdoor mesocosm experiments assessing impacts of two cichlids, *Tilapia galilaea* and *Tilapia aurea*, on plankton from Lake Kinneret, Israel. Laboratory feeding rates of both fish increased for larger particles, reaching maximum values for zooplankton and *Peridinium cinctum*. *Tilapia galilaea* had higher feeding rates on *Peridinium elpatiewsky* and on intermediate-sized nanoplankton. Outdoor mesocosm experiments examining fish impacts on plankton community structure included two 21-d spring and summer experiments of replicated 2 × 2 factorial design (*T. galilaea* × *T. aurea*). Both fish suppressed crustaceans and rotifers. In the spring, fish also suppressed chlorophyll concentration and the dominant phytoplankter *P. cinctum*. In the summer, when nanoplankton dominated the phytoplankton and the smaller *P. elpatiewsky* was the most abundant dinoflagellate, only *T. galilaea* suppressed *Peridinium* spp., while presence of *T. aurea* was associated with increased chlorophyll concentration. Overall, *T. galilaea* suppressed more and enhanced fewer nanoplankton taxa than did *T. aurea*. Production of each fish species was lowest when both species were together, suggesting potential competition for plankton resources.

Les auteurs ont réalisé des essais de broutage sélectif en laboratoire et des essais extérieurs en mésocosme dans le but d'évaluer les incidences de deux cichlidés, *Tilapia galilaea* et *Tilapia aurea*, sur le plancton du lac Kinneret (Israël). Les taux d'alimentation en laboratoire des deux poissons augmentaient avec la grosseur des particules pour atteindre des valeurs maximales avec le zooplancton et *Peridinium cinctum*. Les *Tilapia galilaea* présentaient des taux d'alimentation plus élevés pour *Peridinium elpatiewsky* et le nanoplancton de taille intermédiaire. Les essais extérieurs en mésocosme ayant pour but de déterminer les effets des poissons sur la structure de la communauté planctonique comprenaient deux expériences de 21 d, réalisées au printemps et en été, d'une conception par réplique factorielle 2 × 2 (*T. galilaea*) × *T. aurea*). Les deux poissons ont supprimé les crustacés et les rotifères. Au printemps, les poissons avaient aussi pour effet de supprimer la chlorophylle et l'organisme phytoplanctonique dominant *P. cinctum*. Au cours de l'été, lorsque le nanoplancton dominait le phytoplancton et que *P. elpatiewsky*, plus petit, était le dinoflagellé le plus abondant, seul *T. galilaea* faisait disparaître les espèces de *Peridinium* tandis que la présence de *T. aurea* correspondait à une augmentation de la concentration de chlorophylle. De façon générale, *T. galilaea* supprimait un plus grand nombre de taxons de nanoplancton et en favorisait un nombre plus restreint que ne le faisait *T. aurea*. La production de chacune des espèces de poisson était la moins élevée lorsque les deux espèces étaient présentes ensemble, ce qui porte à croire à une compétition possible pour les ressources planctoniques.

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In Lake Kinneret, Israel, declines in the catch of the Galilee Saint Peter's fish (*Tilapia galilaea* = *Sarotherodon galilaeus*) relative to that of the previously rare blue tilapia (*Tilapia aurea*) were associated with stocking of blue tilapia (Gophen et al. 1983). Based on evidence derived from diet studies and laboratory experiments, we hypothesized that competition between these fish species could have contributed to the reduced catch of *T. galilaea*. Both species are primarily planktivorous, feeding on zooplankton and phytoplankton (Spataru 1976; Spataru and Zorn 1978; Gophen 1980). *Tilapia galilaea* and *T. aurea* larger than 62 and 76 mm standard length (SL), respectively, feed on plankton as pump filter-feeders using rapid nondirected suctions to draw water and food particles into the buccal cavity (Gophen et al. 1983). Smaller fish may filter-feed but also visually locate and attack individual food items. In laboratory feeding experiments, *T. galilaea* and *T. aurea* had similar feeding selectivities for zooplankton (Gophen et al. 1983) but their selectivities for phytoplankton have not been compared.

In this study, we conducted two sets of experiments to assess the impacts of the two cichlid species on plankton from Lake Kinneret, Israel. The first group of laboratory experiments examined selective plankton grazing by the fish, and a second set of outdoor mesocosm experiments examined fish impacts on plankton community structure.

Materials and Methods

Prior to the selective grazing experiments, fish were collected from the lake or acquired from kibbutz Mevo Hama fish rearing ponds and acclimated at the Yigal Allon Kinneret Limnological Laboratory for 1 mo in 60-L plastic tubs continually supplied with water and plankton pumped from the lake. In the selective grazing experiments, fish feeding rates on lake plankton were measured by monitoring declines of phytoplankton and zooplankton densities in the 60-L plastic tubs containing five to eight feeding *T. galilaea* or *T. aurea* (121–142 mm SL). At the beginning of each experiment, the tubs were cleaned and nearshore water containing plankton was pumped into the tubs. To ensure that adequate plankton densities and a full zooplankton species complement were present in each experiment, we supplemented plankton in the tubs with zooplankton collected from the lake with a 63-μm-mesh net. This resulted in initial zooplankton concentrations ranging from 278 to 3780 organisms/L. Plankton were kept in suspension by aeration, movements of the fish, and stirring every 10 min. Feeding trials were conducted at 28.8 ± 0.1°C under combined fluorescent and natural sunlight. After completion of each grazing experiment, fish were transferred to clean tubs with a continual flow of lake water and plankton for 24–48 h.

During each experiment, duplicate water samples were collected from tubs initially and after 60 min of feeding. This was accomplished by stirring each tub and then quickly lowering a 6-cm-diameter Plexiglas tube onto a randomly placed rubber stopper on the tub bottom (Drenner et al. 1982). Zooplankton were removed from one sample with a 63-μm-mesh sieve and preserved in 5% formalin. Phytoplankton in 125 mL of unfiltered water from the second sample were preserved by addition of 1% Lugol's solution. Zooplankton and phytoplankton were counted with a dissecting microscope and inverted compound microscope, respectively. Phytoplankton taxa included in the analyses were those that occurred at densities ≥75 organisms/mL and that were present in at least two of the five feeding trials conducted for each fish species. We corrected for particle loss due to zooplankton feeding by monitoring the loss of phytoplankton and zooplankton from fishless control tubs. Feeding rate constant (k) was calculated as

$$k = (D_1 - D_2)/G$$

where G is grams of fish per litre and D_1 and D_2 are instantaneous particle loss rates in tubs with and without fish, respectively, calculated as

$$D = -\ln(P_f/P_i)/T$$

where P_i and P_f are initial and final plankter densities (organisms per millilitre) and T is the experiment duration (hours) (Dodson 1975).

The mesocosm experiments examined the impacts of both fish species on lake plankton in a linear array of eight cylindrical 5-m³ black plastic tanks (1.9 m in diameter). Because the Lake Kinneret phytoplankton community alternates between *Peridinium* dominance in winter and spring and nanoplankton dominance during the summer and fall (Pollingher and Berman 1978), we conducted experiments during spring and summer. Experiments of 3 wk duration began 24 March and 5 August 1985.

Experiments were of 2 × 2 factorial design (presence or absence of *T. galilaea* × presence or absence of *T. aurea*), allowing us to examine main and interaction effects of the fish species. Two replicates of each treatment combination (no fish, *T. galilaea*, *T. aurea*, and *T. galilaea* + *T. aurea*) were assigned to tanks using a randomized block design. Blocks consisted of tanks 1–4 and tanks 5–8.

Prior to the mesocosm experiments, fish were collected from the lake or acquired from kibbutz Mevo Hama fish rearing ponds and acclimated at the Yigal Allon Kinneret Limnological Laboratory for at least 4 d in outdoor holding tanks prior to each experiment. Holding tanks continually received water and plankton pumped from the lake. Before stocking and after experiments, fish were individually weighed and measured. The spring and summer experiments utilized, respectively, 6 or 5 per tank of each fish species in single species treatments, and 12 or 10 fish, in the treatment combination having both *T. galilaea* and *T. aurea*. Average fish biomass for each species group added to the tanks was 620.5 g (range 606.4–637.5 g, mean SL = 140 mm) in the spring experiment and 610.1 g (range 601.1–615.9 g, mean SL = 151 mm) in the summer experiment. No fish mortality occurred during the experiments.

Double biomass was used in tanks containing both fish species and was necessary to hold the impact of each fish species constant within the context of the factorial design. Also, the equivalent density and biomass of each fish species in the presence or absence of each other holds intraspecific competition constant while allowing interspecific competition to vary (Werner and Hall 1977, but see Maiorana 1977). Although the biomass levels used in our experiments may exceed those likely to be observed for these species in Lake Kinneret, they were selected for these experiments to allow assessment of community responses during relatively short-term experiments while minimizing container effects. We feel that the community responses seen are qualitatively similar to impacts expected from fish in the lake although they may differ in magnitude.

Tanks were filled at the beginning of each experiment with water pumped from approximately 30 m offshore at a depth of 1.5 m. In the first experiment, no water was added to the tanks after initial filling. Because additional experiments (not presented here) indicated that continuous addition of lake water reduced diel fluctuations of dissolved oxygen, the tank system

was modified before the second experiment to provide continuous addition of approximately 1 L lake water/min to each tank. Previous experiments indicated that temperatures in the tanks differed by less than 4° from values measured in a nearby cove of the lake.

After filling the tanks, we supplemented zooplankton in them with plankton collected 1–2 km offshore. Sixteen 10-m vertical hauls using a 300-μm-mesh, 44-cm-diameter conical plankton net were pooled and equal portions of the composite added to each tank. This assured the presence of limnetic forms in all tanks.

Tanks were mixed for 2 h daily by an air-lift mixer system. Air from a blower was injected 1 m below the water surface into 5-cm-diameter open plastic pipes suspended from floats in each tank. Mixers moved a water volume equivalent to tank volume in approximately 1 h, destratifying and aerating the tanks. Because *Peridinium* divide primarily at night and are sensitive to agitation during this period (Pollingher and Serruya 1976), we operated mixers only during the day.

Tanks were sampled initially and twice weekly for 3 wk. Mean tank water temperatures ranged from 17 to 22°C and from 28 to 32°C during the spring and summer experiments, respectively. On each sampling date, a single composite water sample was produced for each tank by mixing five replicate samples collected with a 2.5-m-long, 1.5-cm-diameter plastic pipe lowered to within 10 cm of the tank bottom. Aliquots were taken for analysis of chlorophyll, water chemistry, and phytoplankton. For each sample, we made triplicate fluorometric chlorophyll determinations on 90% acetone extracts of material retained by a glass fiber filter (0.8–1.0 μm pore size) (APHA 1981). Fluorimeter readings were calibrated by comparison with spectrophotometric chlorophyll determinations (Strickland and Parsons 1972; D. Wynne, Yigal Allon Kinneret Limnological Laboratory, P.O. Box 345, Tiberias, Israel, pers. comm.).

Water chemistry analyses were performed for the spring experiment by the Mekorot Watershed Unit, Kinneret Limnological Laboratory, using standard methods (APHA 1981; Nydhal 1978; Solorzano 1969). Parameters examined included total phosphorus, total dissolved phosphorus, soluble reactive phosphorus, total Kjeldahl nitrogen, ammonia, nitrite, and nitrate.

Phytoplankton were preserved by adding 1% Lugol's iodine solution and settled overnight before counting with an inverted microscope. Dinoflagellate densities for each tank were determined by counting all cells in a 1-mL subsample, while densities of other algal taxa were determined by counting 20% of the material settled from 10-mL samples.

Zooplankton samples were collected from each tank at the conclusion of the mixing period with single vertical hauls of a 63-μm-mesh, 16-cm-diameter net. At least 200 organisms or one third the total sample volume from each tank was counted using a dissecting microscope.

Data from the community response experiments were analyzed using a multivariate profile analysis of repeated measures (Wilkinson 1984; Winer 1971). This procedure sums data across sampling dates into a univariate test to detect treatment effects (Wilkinson 1984). Statistical significance was inferred at the $P < 0.1$ level.

Results

In the selective grazing experiments, feeding rates of *T. galilaea* increased with particle size, leveling when particles exceeded 500 μm³ (Fig. 1). *Tilapia galilaea* had maximum feeding rates on zooplankton, *Peridinium cinctum*, and some intermediate-sized nanoplankton. Feeding rates of *T. aurea* also increased with particle size, with highest feeding rates on *P. cinctum* and zooplankton. *Tilapia aurea* had lower feeding rates than *T. galilaea* on *Peridinium elpatiewsky* and intermediate-sized nanoplankton. Neither fish efficiently grazed the smallest nanoplankton.

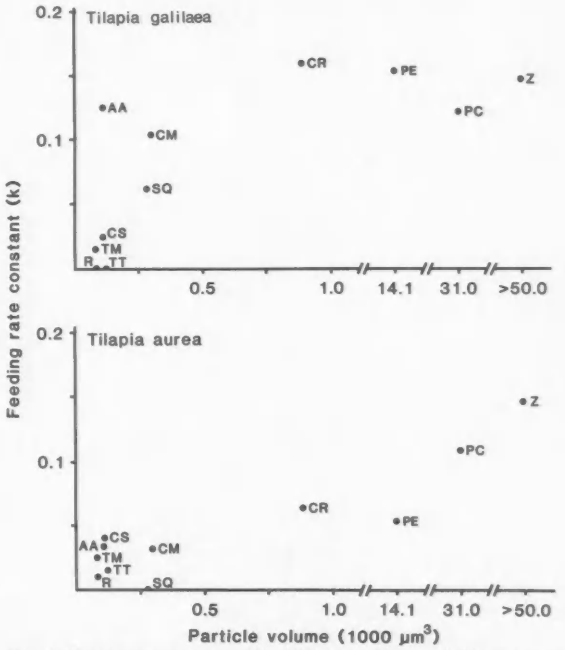

FIG. 1. Feeding rate constants (k) of *Tilapia galilaea* and *Tilapia aurea* (litres per gram of fish per hour) for zooplankton (Z), *Peridinium cinctum* (PC), *Peridinium elpatiewsky* (PE), *Cryptomonas reflexa* (CR), *Scenedesmus quadricauda* (SQ), small *Cyclotella* sp. (CS), *Tetraedron minimum* (TM), *Tetraedron trigonum* (TT), and *Rhodomonas minuta* v. *nannoplan* (R).

In the spring mesocosm experiment, presence of *T. galilaea* was associated with reduced densities of *Ceriodaphnia* spp., adult *Mesocyclops* sp., copepodids, copepod nauplii (Fig. 2), *Peridinium* spp., and *Cyclotella* spp. (Fig. 3) and total phosphorus concentration (Table 1) and chlorophyll concentration (Fig. 3). Presence of *T. galilaea* was also associated with increased densities of *Acronema augustum*, *Tetraedron* spp., and *Chrysochromulina* spp. (Fig. 3) and increased concentration of dissolved inorganic nitrogen (Table 1). Presence of *T. aurea* was associated with reduction in densities of *Ceriodaphnia* spp., *Bosmina* spp., adult *Mesocyclops* sp., copepodids, copepod nauplii, *Keratella* spp. (Fig. 2), and *Peridinium* spp. and chlorophyll concentration (Fig. 3). Increased nanoplankton densities associated with *T. aurea* include those of *Coelastrum microporum*, *Cyclotella* spp., *Golenkenia radiata*, *A. augustum*, *Rhodomonas minuta* v. *nannoplan*, *Tetraedron* spp., and *Chrysochromulina* spp. (Fig. 3). Because nanoplankton species accounted for only a small fraction of the total algal biomass in this experiment, their enhancement in the presence of either fish species did not offset reductions in chlorophyll concentration due to *Peridinium* suppression.

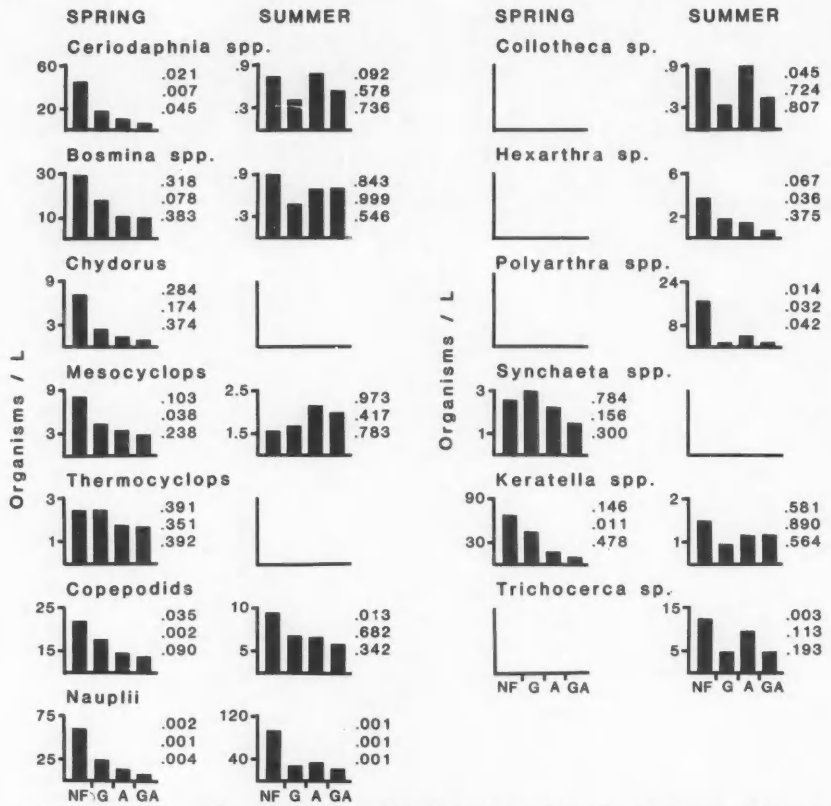

FIG. 2. Mean numbers of zooplankton in no fish (NF), *Tilapia galilaea* (G), *Tilapia aurea* (A), and *T. galilaea* + *T. aurea* (GA) treatment combinations for the spring and summer experiments. Probability values for *T. galilaea* (upper) and *T. aurea* (middle) main effects and interactions (lower) are given to the right of each histogram. Evaluation of *T. galilaea* or *T. aurea* main effects from this figure and Fig. 3 can be achieved by comparing the mean values for treatments containing the species (G and GA for *T. galilaea*, A and GA for *T. aurea*) with those from which it is absent (NF and A for *T. galilaea*, NF and G for *T. aurea*).

TABLE 1. Mean values of water chemistry parameters (mg/L) in replicate tanks during the spring experiment. Treatments were no fish (NF), *T. galilaea* (G), *T. aurea* (A), and *T. galilaea* + *T. aurea* (GA). Probability values for *T. galilaea* (Tg) and *T. aurea* (Ta) main effects and interactions (Tg × Ta) are given.

	Treatment combinations				Probability values		
	NF	G	A	GA	Tg	Ta	Tg × Ta
Phosphorus							
Total dissolved	0.013	0.016	0.017	0.013	0.919	0.663	0.028
Orthophosphate	0.006	0.007	0.008	0.008	0.542	0.514	0.571
Total	0.063	0.053	0.048	0.042	0.022	0.005	0.538
Nitrogen							
Nitrite	0.012	0.017	0.017	0.020	0.004	0.004	0.237
Nitrate	0.124	0.210	0.228	0.250	0.019	0.007	0.083
Ammonia	0.023	0.128	0.127	0.297	0.001	0.001	0.072
Total	1.094	1.089	0.954	1.059	0.194	0.057	0.162

In the summer experiment, presence of *T. galilaea* was associated with reduced densities of *Ceriodaphnia* spp., copepodids, copepod nauplii, *Collotheca* spp., *Hexarthra* sp., *Polyarthra* spp., *Trichocerca* spp. (Fig. 2), *Peridinium* spp., *Chroococus turgidus*, *G. radiata*, *Chodatella citriformis*, and *A. augustum* and increased densities of *R. minuta* v. *nannoplan* (Fig. 3). Presence of *T. aurea* was associated with reduced densities of copepod nauplii, *Hexarthra* sp., *Polyarthra*

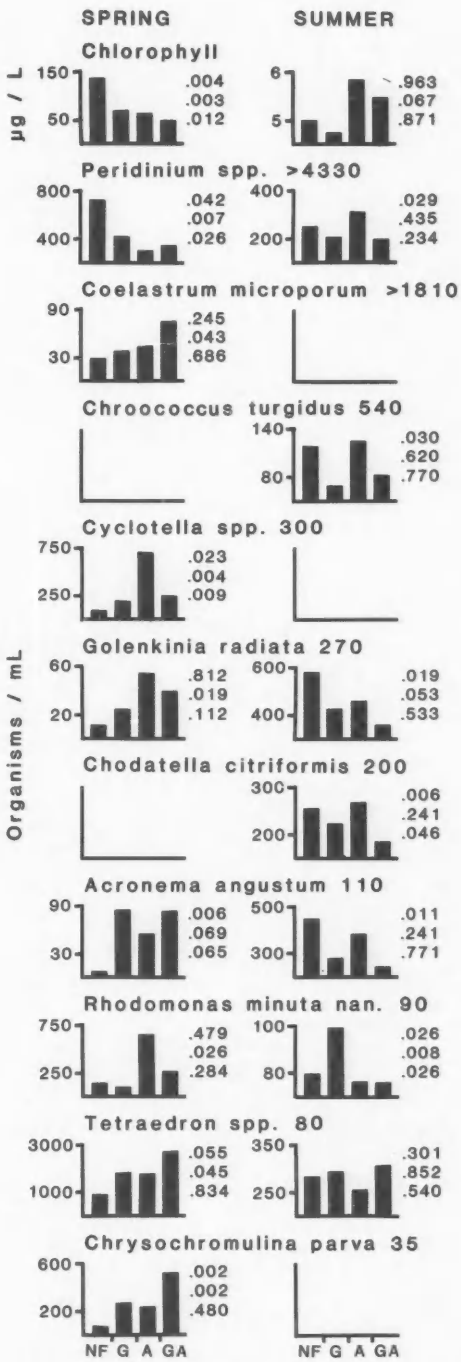

FIG. 3. Mean chlorophyll concentration (µg/L) and mean phytoplankton densities in no fish (NF), *Tilapia galilaea* (G), *Tilapia aurea* (A), and *T. galilaea* + *T. aurea* (GA) treatment combinations for the spring and summer experiments. Phytoplankton volume (µm³) is given to the right of each genus name. Probability values for *T. galilaea* (upper) and *T. aurea* (middle) main effects and interactions (lower) are given to the right of each histogram.

spp. (Fig. 2), *G. radiata*, and *R. minuta* v. *nannoplan* and increased chlorophyll concentration (Fig. 3). Total chlorophyll concentrations were much lower during the summer experiment than during the spring.

Significant interaction effects were observed infrequently in our data, suggesting that in most cases the effects of the two fish species were independent and additive. Interactions between main effects for zooplankton were observed for two groups (nauplii and copepodids) in the spring experiment (Fig. 2) and three (*Ceriodaphnia* sp., nauplii, and *Polyarthra* sp.) in the summer (Fig. 2). Phytoplankton taxa showed two significant interactions (*C. turgidus* and *P. cinctum*) in the spring experiment (Fig. 3) and two significant interactions (*C. citriformis* and *R. minuta* v. *nannoplan*) in the summer experiment (Fig. 3). Potential mechanisms for producing interaction effects in tank experiments may include behavioral interactions between fishes, or between fish and zooplankton, or nonlinear biomass effects on specific parameters.

In the spring experiment, fish biomass increased in all tanks. When alone, biomass of *T. galilaea* and *T. aurea* increased by 9.9 and 4.0% ($t = 3.497$, $P = 0.002$), respectively. In the treatment combinations with both fish, biomass increased and by 3.3% for *T. galilaea* and by 0.7% for *T. aureus* ($t = 3.174$, $P = 0.005$). Both species exhibited significantly reduced growth rates in the presence of the other species ($t = 3.830$, $P = 0.001$ for *T. galilaea* and $t = 3.636$, $P = 0.002$ for *T. aurea*).

During the summer experiment, fish biomass declined in all treatments. When alone, biomass of *T. galilaea* declined by 5.6%, while that of *T. aurea* decreased by 9.2% ($t = 2.084$, $P = 0.054$). *Tilapia galilaea* biomass decreased by 14.3% and *T. aureus* biomass by 5.3% ($t = 4.317$, $P = 0.001$) in the treatment with both fish. Both fish species had significantly greater weight loss in the presence of the other species ($t = 4.094$, $P = 0.002$ for *T. galilaea* and $t = 2.3265$, $P = 0.036$ for *T. aurea*).

Discussion

In the mesocosm experiments, *T. galilaea* and *T. aurea* had similar effects on zooplankton, suppressing several crustacean and rotifer taxa while enhancing none. Both fish suppressed *Peridinium* in the spring when *P. cinctum* (31 000 µm³/cell) was the dominant dinoflagellate species. In the summer, when the smaller dinoflagellate *P. elpatiewsky* (14 000 µm³/cell) was the most abundant large algal species, only *T. galilaea* suppressed *Peridinium* sp. Overall, *T. galilaea* suppressed more and enhanced fewer nanoplankton taxa than did *T. aurea*. These results are consistent with feeding trials which showed that both fish had maximum feeding rates on zooplankton and *P. cinctum*, but *T. galilaea* had higher feeding rates than *T. aurea* on *P. elpatiewsky* and on intermediate-sized nanoplankton. Other studies (Drenner et al. 1984a, 1984b, 1986, 1987) have shown direct suppression of zooplankton and phytoplankton by omnivorous filter-feeding fish and have suggested that such fish may indirectly enhance phytoplankton through their effects on nutrients and zooplankton predation. Small nanoplankton species that are inefficiently grazed by these fish might be especially sensitive to such enhancement.

Our data from the spring experiment indicate that the fish can significantly increase the dissolved nitrogen fraction in the tanks, chiefly through increases in ammonia and nitrate, without having significant impacts on the total nitrogen present. A

reversal of these effects was observed with phosphorus. Tanks containing fish had significantly reduced total phosphorus concentrations without significant effects on the dissolved fractions. We suspect that the increase in dissolved nitrogen compounds reflects the metabolic release of ammonia by the fish. The decrease in phosphorus probably results from loss through sedimentation of fecal pellets which are tightly bound and sink rapidly to the bottom of the tanks. Other experiments (not presented here) indicate that fish substantially increased sedimentation rates through feces production.

The direct and indirect effects of these two cichlids on phytoplankton have potentially important implications for water quality of Lake Kinneret (Serruya et al. 1980) which annually provides 35% of Israel's freshwater. *Peridinium* accounts for over 80% of the annual algal biomass in Lake Kinneret (Serruya et al. 1979). Because *T. galilaea* has been considered the most important consumer of *Peridinium*, Serruya et al. (1980) suggested that *Peridinium* abundance and levels of suspended and dissolved organic materials might be suppressed by increasing the density of *T. galilaea* in the lake. In the mesocosm experiments reported here, *T. galilaea* suppressed total chlorophyll levels when *Peridinium* was abundant in the spring, but did not significantly affect chlorophyll levels in the summer experiment when *Peridinium* densities and algal biomass were much lower. While it is possible that the use of the continuous inflow in the summer experiment contributed to these seasonal differences, other experiments (not reported here) showed that *T. galilaea* continues to suppress algal abundance when *Peridinium* is dominant and the tanks are receiving continuous inflow.

Because it has been suggested that *T. galilaea* utilizes *Peridinium* more intensively than *T. aurea* in the lake, Gophen et al. (1983) expressed concern that displacement of *T. galilaea* by *T. aurea* could alter algal population dynamics and result in increased algal abundance and reduced water quality in the lake. While we find few differences between the fish in their ability to consume *P. cinctum*, differences in grazing efficiency on intermediate- and small-sized phytoplankton could be important, especially during the nanoplankton period.

Although *T. aurea* did show somewhat greater reductions in *Peridinium* and chlorophyll in the spring experiment, *T. galilaea* grew significantly more. During the summer experiment, both fish species lost weight, and *T. aurea* lost significantly more than did *T. galilaea*. Although several factors may have contributed to these results, they could reflect a higher food conversion efficiency for *T. galilaea* as suggested by Spataru (1976).

Despite the difference in algal grazing efficiencies of the two cichlids, they exhibit a high degree of overlap in resource utilization, particularly for zooplankton and *P. cinctum*. Although our experiments permitted only limited behavioral flexibility and may thus have increased apparent niche overlap (Werner and Hall 1976), the data support the conclusions of Gophen et al. (1983) that stocking of *T. aurea* could divert resources from *T. galilaea* in Lake Kinneret.

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Secondary Production of Tiger Salamanders (*Ambystoma tigrinum*) in Three North Dakota Prairie Lakes

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Deutschman, M. R., and J. J. Peterka. 1988. Secondary production of tiger salamanders (*Ambystoma tigrinum*) in three North Dakota prairie lakes. Can. J. Fish. Aquat. Sci. 45: 691–697.

In three prairie lakes studied in 1981–82, larval tiger salamander (*Ambystoma tigrinum*) densities reached highs of 5000·ha^{-1}, maximum biomass (wet weight) was 180 kg·ha^{-1}, and maximum annual production was 565 kg·ha^{-1}. Within a given lake, overwinter survival of larvae varied markedly from year to year. Overwinter survival of larvae was excellent in Lake II; in spring 1981, densities were 800–1000·ha^{-1}. In 1982, no larvae overwintered in Lake II, and none overwintered in Lake I in either 1981 or 1982. In May 1981, larvae were large (mean weight of 150 g) and their biomass of 150 kg·ha^{-1} was nearly as large as the maximum biomass of 160 kg·ha^{-1} in late July of larvae from the year's cohort. This was in contrast with 1982 in Lake II and with both 1981 and 1982 in Lake I when there was an extremely low biomass in the spring, composed of many small larvae. The large larvae that overwintered in Lake II in 1981 did not prey on young-of-the-year larvae; their high densities may have actually reduced invertebrate predation on salamander larvae; in July, larval salamander densities of 5000·ha^{-1} were the highest measured.

Les auteurs ont trouvé, pour trois lacs de prairie étudiés en 1981–1982, que les densités de larves de salamandre tigre (*Ambystoma tigrinum*) atteignaient des maximum de 5 000·ha^{-1}, que la biomasse maximale (poids humide) était de 180 kg·ha^{-1} et que la production annuelle moyenne atteignait 565 kg·ha^{-1}. Au sein d'un lac donné, le taux de survie des larves après un hiver variait de façon marquée d'une année à l'autre. Ce taux était excellent dans le lac II où les densités au printemps de 1981 atteignaient 800–1 000 larves·ha^{-1}. En 1982, aucune larve n'a survécu à l'hiver dans ce lac et aucune larve n'a survécu aux hivers de 1981 et 1982 dans le lac I. En mai 1981, les larves étaient grosses (poids moyen de 150 g) et leur biomasse de 150 kg·ha^{-1} était presque aussi élevée que la biomasse maximale de 160 kg·ha^{-1} notée vers la fin de juillet pour les larves de la cohorte annuelle. Cela contrastait avec les résultats obtenus pour 1982 dans le lac II et pour 1981 et 1982 dans le lac I où la biomasse de printemps était extrêmement faible et surtout représentée par un grand nombre de petites larves. Les grosses larves ayant survécu à l'hiver dans le lac II en 1981 ne s'attaquaient pas aux larves nées pendant l'année; les fortes densités ont même pu contribuer à réduire la prédation des larves par les invertébrés. En juillet, la densité des larves de salamandre atteignait 5 000 individus·ha^{-1}, la valeur la plus élevée notée.

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Beginning in the 1970's, considerable research was conducted to determine the feasibility of growing rainbow trout (*Salmo gairdneri*) in prairie lakes in Manitoba, Saskatchewan, and Alberta (Lawler et al. 1974) and in North Dakota (Myers and Peterka 1976). Results from these studies indicated that tiger salamanders (*Ambystoma tigrinum*) were abundant in prairie lakes in North Dakota (Myers and Peterka 1976) and in Manitoba (Olenick and Gee 1981; Tavarutmaneegul 1978). However, no studies were conducted to provide data on seasonal densities, biomass, or secondary production of salamanders. Based on a 2-yr study from 1981 to 1982, we provide such data for three North Dakota prairie lakes. Such information could have implications for the aquaculture of fishes. We hope to draw attention to the role salamanders play as important components of wetland communities and to provide information on secondary production in prairie lakes in the prairie pothole region of central North America. This region extends from south-central Canada to the north-central United States and produces 50% of the North American duck crop in an average year (Tiner 1984).

In general, salamanders have been overlooked in most wetland studies in the prairie pothole region. Although quantitative data are lacking, salamanders probably play an important role in structuring invertebrate communities and in the energetics of prairie lake ecosystems. Food habit studies indicate that they consume primarily cladocerans, chironomids, and amphipods (Deutschman 1984). Olenick and Gee (1981) and Myers (1973) found considerable overlap in the diet of tiger salamanders and rainbow trout in prairie lakes in Manitoba and North Dakota;

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TABLE 1. Numbers of and sex ratios (m = male; f = female) of tiger salamanders (A = adult; L = larvae) captured in two North Dakota prairie lakes, 1982.

	Lake I			Lake II		
Date	Form	Number captured	Sex ratio (m:f)	Form	Number captured	Sex ratio (m:f)
1 May	A	71	6:1	A	45	4:1
22 May	A	73	4:1	A	12	2:1
25 June	A	6		A	2	
	L	155		L	0	
7–9 July	A	9		A	3	
	L	1134		L	302	
20–21 July	A	2		A	5	
	L	757		L	890	
3–5 Aug.	A	0		A	0	
	L	326		L	511	
17–18 Aug.	A	47		A	3	
	L	577		L	594	
31 Aug.	—	—		A	4	
				L	144	
18 Sept.	A	0		A	7	
	L	141	1:1	L	396	1:2
27 Oct.	L	51	1:2	L	88	1:1

TABLE 2. Petersen population estimates for tiger salamander adults and larvae in Lake I and Lake II, 1982. Numbers in parentheses are 95% C.L. for *N*; *R* was treated as a poisson variable.

Estimate for date/period	Form	Number in census sample (*C*)	Number recaptured (*R*)	Total number marked (*M*)	Estimate (*N*)
Lake I					
1 May	Adult	73	29	68	170 (120–250)
22 May	Adult	6	3	38	68 (28–170)
7–8 July	Larvae	1077	31	1124	37 898 (26 284–55 076)
20–21 July	Larvae	695	29	736	17 098 (12 041–25 145)
3–5 Aug.	Larvae	718	32	281	6144 (4400–8890)
17–18 Aug.	Larvae	192	8	522	11 215 (6008–22 941)
18 Sept.	Larvae	51	1	131	3432 (1040–6240)
Lake II					
1 May	Adult	12	4	32	86 (38–214)
13 July	Larvae	1652	18	186	16 269 (10 514–26 420)
20–21 July	Larvae	1645	109	869	13 018 (10 808–15 668)
3–5 Aug.	Larvae	1134	82	487	6673 (5388–8254)
17–18 Aug.	Larvae	628	36	577	9826 (7157–13 930)
31 Aug.	Larvae	396	7	99	4963 (2578–11 447)
18 Sept.	Larvae	88	8	386	3827 (2050–7828)

amphipods (*Gammarus lacustris*) comprised 78 and 30% by volume of stomach contents of salamanders and trout, respectively (Myers 1973). The diets of fathead minnows (*Pimephales promelas*) and brook sticklebacks (*Culaea inconstans*), species that commonly occur with salamanders, have considerable overlap with the diets of salamanders. Crustaceans (cladocerans, copeopods, and amphipods) comprised 94% by weight of food items found in 541 fathead minnow stomachs and 95% by weight of food items found in 30 brook stickleback stomachs in North Dakota wetlands that also contained populations of tiger salamanders (Held and Peterka 1974). Salamanders may influence the availability of invertebrates used by ducks. Some ducks rely heavily on chironomids and amphipods; lesser scaup relied heavily on amphipods during the breeding and mating season (Swanson and Nelson 1970). Salamanders are an important food for predacious birds and mink (Wiedenheft 1983).

The subspecies *Ambystoma tigrinum melanostictum* is found throughout the prairie pothole region, except for the northeastern part of North Dakota and northward into Manitoba and Saskatchewan where the subspecies is *Ambystoma tigrinum diaboli* (Wheeler and Wheeler 1966). Larvae found in North Dakota are among the largest recorded for these subspecies. Larson (1974) recorded a maximum total length of 38.5 cm for a larval *A. t. diaboli* in Devils Lake, and Wiedenheft (1983) recorded a maximum length of 30.0 cm for a larval *A. t. melanostictum* in a prairie lake in Stutsman County.

Salamanders are most successful in prairie lakes that are shallow (maximum depths not exceeding 5 m) and do not support populations of predacious fishes. Winterkills of larval salamanders and of fishes are common, and the collapse of blue-green algae blooms may cause summerkills. Salamanders are also successful in saline waters; we collected adult and larval

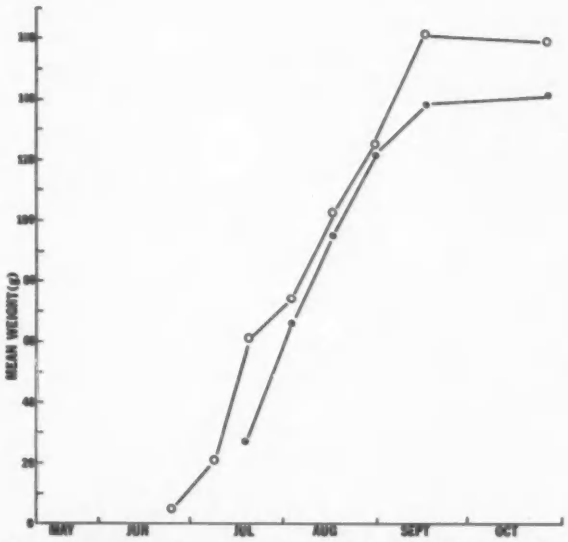

FIG. 1. Mean wet weight of larvae in Lake I (open circles) and in Lake II (solid circles) in 1982.

tiger salamanders from West Stump Lake, North Dakota, where water conductivity was 12 500 $\mu S \cdot cm^{-1}$ (J. J. Peterka, pers. obs.).

Lake Descriptions

Two Stutsman County, North Dakota, lakes (44°04′N, 99°05′W) were studied in 1981 and 1982. Lake I had a maximum depth of 2.4 m and an area of 24 ha. Lake II had a maximum depth of 1.7 m and an area of 10 ha. Water levels dropped 0.3 m from June to September in both 1981 and 1982. Water temperatures from April to May in 1981 and 1982 were similar; maximum water temperatures of 28°C occurred in mid-July. Mean dissolved oxygen concentrations for July–August 1982 in Lake II and in August in Lake I were only 4 and 5 $mg \cdot L^{-1}$, respectively. Lows of 2.8 mg dissolved $oxygen \cdot L^{-1}$ were measured in both lakes. The mean specific conductance of lake waters from May to October was 1600 $\mu S \cdot cm^{-1}$ (25°C) in Lake I (maximum of 1740 in September) and 1200 $\mu S \cdot cm^{-1}$ in Lake II (maximum of 1380 in September). Secchi disc measurements averaged 1.5 m from July to October with minimums of 0.5 m, and they were about 1.7 m during much of July and August (some readings were to the lakes' bottom). No fish were present in Lake I or II whereas in Lake III, fathead minnows were abundant. Lake III, also in Stutsman County (47°15′N, 99°12′W), had a maximum depth of 2.1 m and an area of 10 ha.

Bullrushes (*Scirpus* spp.) were common along shorelines in Lake I, and sago pondweed (*Potamogeton pectinatus*) occupied open-water areas. Cattails (*Typha* spp.) and bullrushes were the dominant emergent plant species in Lake II, and coontail (*Ceratophyllum demersum*) and water milfoil (*Myriophyllum exalbescens*) were the dominant submergent plants in open-water areas. *Gammarus lacustris* and *Hyalella azteca*, chironomids, *Chaoborus*, and *Daphnia* were the most numerous invertebrates (Deutschman 1984).

Methods

To estimate their numbers, we trapped adults and larvae with frame nets, setting four or five nets on various dates from 1 May to 27 October in Lakes I and II. Nets were set at sunset and emptied the following morning. Net frames were 1 × 2 m, leads were 10–15 m long, and mesh sizes (bar measure) were 0.6 and 1.3 cm. Salamanders were marked by removing all phalanges of one foot. This was necessary because early attempts to remove only one or two phalanges resulted in rapid regrowth. To monitor any effects of marking, 10 marked salamanders for each sampling date in each lake were placed in a live cage. Complete regrowth of all phalanges occurred after about 8 wk, and only 1 of 240 individuals placed in the cages died. Marked animals were returned toward the lake's center at least 20 m from the capture point.

We used the modified Petersen method, $(m + 1)(c + 1)/(r + 1)$ where m = the number of animals marked, c = the number of animals in the census sample, and r = the number of recaptures in the census sample, to estimate population size (Ricker 1975). The census sample consisted of all salamanders captured during the 8 wk that the marks could be reliably dis-

TABLE 3. Numbers, mean wet weight, and biomass of tiger salamanders in Lake I and Lake II (A = adult; L = larvae), 1982. Numbers of individuals used to obtain mean weight are in parentheses.

Lake I					Lake II				
Date	Form	Petersen estimate (no.·ha^{-1})	Mean weight (g)	Biomass (kg·ha^{-1})	Date	Form	Petersen estimate (no.·ha^{-1})	Mean weight (g)	Biomass (kg·ha^{-1})
					1 May	A	9	71.3 (35)	0.7
1 May	A	7	83.7 (71)	0.6	31 May	L	3473[a]	0.004[c]	0.02
22 May	A	3	83.8 (33)	0.3	8 July	L	1600[b]	7.4 (76)	11.8
31 May	L	3253[a]	0.004[c]	0.01	13 July	L	1627	17.1 (11)	27.8
7 July	L	1579	20.9 (206)	33.0	20 July	L	1302	27.5 (51)	35.8
20 July	L	712	61.4 (144)	43.7	3 Aug.	L	960[b]	66.0 (145)	63.4
3 Aug.	L	512[b]	74.6 (76)	38.2	17 Aug.	L	740[b]	95.3 (30)	70.5
17 Aug.	L	467	102.8 (30)	48.0	31 Aug.	L	496	122.5 (10)	60.8
18 Sept.	L	143	161.8 (30)	23.1	18 Sept.	L	383	139.1 (30)	53.3
31 Oct.	L	100[b]	158.8 (30)	15.9	31 Oct.	L	300[b]	142.1 (30)	42.6

[a]Product of mean number of eggs per female and number of females present on 1 May.
[b]Numbers estimated from smoothed curve in Fig. 3.
[c]Mean ovum weight.

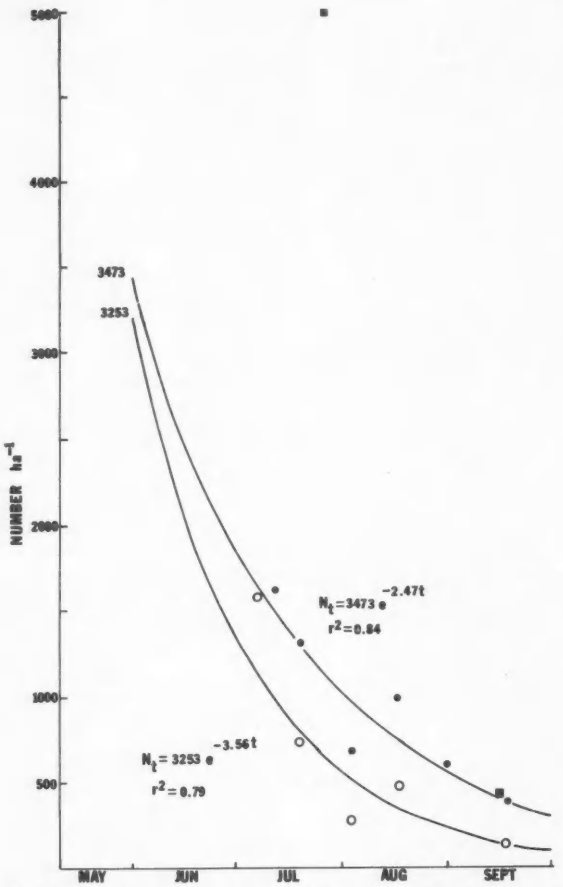

FIG. 2. Larval salamanders in Lake I (open circles) and in Lake II (solid circles) in 1982. Data were fitted (lines) to the equation $N_t = N_0 e^{-zt}$ where N_t = numbers at time t, N_0 = numbers on 31 May (approximate hatching date), z = instantaneous rate of mortality, and t = time (t = 1 from 31 May to 30 September). r^2 is the coefficient of determination. Solid squares (two points) are numbers of larvae in Lake II in 1981.

tinguished. At each netting, we weighed a known number of larvae en masse to obtain an estimate of their mean weight and measured the total length of 30 larvae. For adults, the total length and weight were measured for each individual. Sex was determined by cloacal examination.

Results

Life History

Based on our observations in 1982 and Wiedenheft's in 1981, adult salamanders overwintered on land. Immigration to prairie lakes began in early April, soon after ice-out, and continued until mid-May. We did not find any larvae that overwintered in Lakes I and II in the spring of 1982 whereas Wiedenheft (1983) reported that abundant larvae overwintered in Lake II. In May 1981, larvae comprised 91% of the salamanders caught and adults comprised 9%. No larvae overwintered in Lake I in 1981.

Reproduction began in early May. At this time, adult males outnumbered females by 4–6:1; by late May the ratio decreased to 2–4:1 (Table 1). Eggs were laid throughout May on vegetation or sticks and hatched 20–30 d later (Wiedenheft 1983). Larval numbers declined markedly throughout the summer (Table 2). Few adults were captured after May, probably reflecting emmigration.

By mid-August, most larvae had reabsorbed the fleshy tail and gills and begun metamorphosis. Sex ratios were nearly 1:1 (males to females) in September–October (Table 1). Newly metamorphosed individuals were captured routinely by mid- to late August and were frequently seen on nearby roads during or after a rain. These metamorphs and adults (sexually mature) presumably spent the winter beneath vegetation or the soil (Semlitsch 1983). We captured only seven adults while sampling in the lakes in mid-September and none by late October (Table 1).

Growth

Growth rates of larvae in 1982 were similar in both lakes from June–July to early September; mean weights increased linearly at 1.9 g·d^{-1} (Fig. 1). By mid-September 1982, mean wet weights of larvae were 139 g in Lake II and 162 g in Lake I. In 1981 the mean weight of larvae by mid-September was only 67 g in Lake II and 112 g in Lake I, significantly less ($P < 0.05$) than in 1982. Standard errors of the mean for sample sizes of 20–40 did not exceed 7.0 g (Wiedenheft 1983). No larvae were collected in Lake II until 8 July 1982, indicating an earlier hatch in Lake I, the larger of the two lakes, where larvae were first collected on 25 June. We observed a concentration of adult salamanders in a shallow bay of Lake I in May 1982 which may explain earlier reproduction and larger initial sizes of larvae there, as water temperatures were likely warmer than in the main lake. The mean wet weight of adults on 1 May 1982 was 83.7 g in Lake I and 71.3 g in Lake II (Table 3); their mean total lengths were 25.7 and 24.4 cm, respectively. By early July, mean wet weights of adults had increased to 117.6 g in Lake I and 96.0 g in Lake II.

The length–weight relationship for larvae in both lakes in 1982 could be explained by the parabolic power function $W = aL^b$. This equation explained 99.5% of regression variance ($r^2 = 0.995$); $a = 0.014$, $b = 2.895$, W = wet weight (grams), and L = total length (centimetres) (Deutschman 1984).

Population Estimates

We obtained several estimates of numbers of larvae during June–September and of adults in May (Table 2). Because adults apparently emmigrated from the lakes following reproduction, estimates of their densities are those for breeding populations.

We estimated the numbers of larvae hatched at the end of May (the expected time of hatching) by multiplying the mean number of eggs (1754 ± 177 SE of the mean) in two female salamanders collected just prior to spawning on 22 May by the estimated (Petersen estimates) number of females present on 1 May (Table 3).

The die-off curves (exponential model of Ricker 1975) for larvae were similar in both lakes (Fig. 2); there was a rapid decline in numbers following hatching. Based on these curves, the densities of larvae in Lake I were generally 300–400 larvae·ha^{-1} fewer than in Lake II.

Based on two estimates made by the Schnabel method in Lake II in 1981 (Wiedenheft 1983) the density of larval salamanders on 27 July was extremely high, 4995·ha^{-1} (95% C.L. of 3860–7150), but by 15 September the density of 422·ha^{-1}

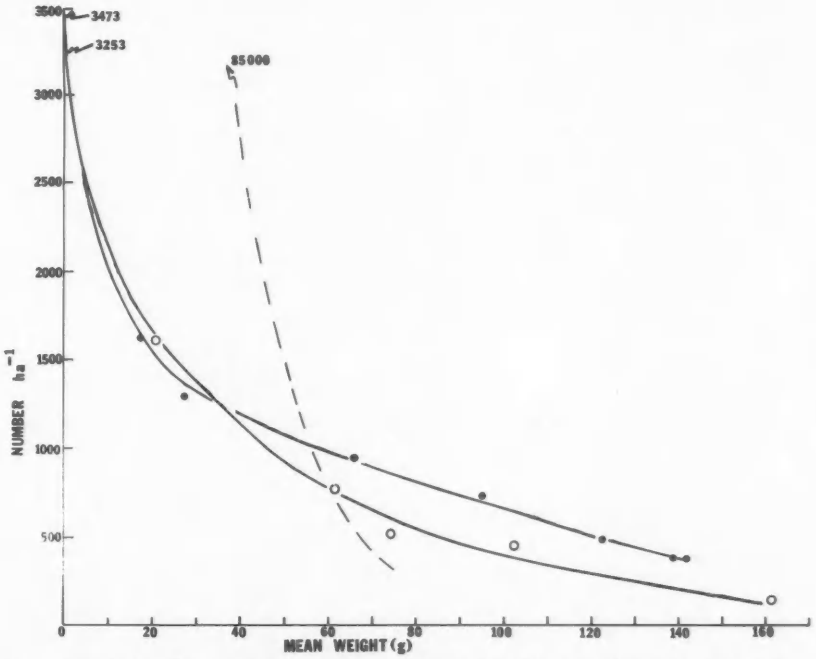

FIG. 3. Larval salamanders and their mean wet weights in Lake I (open circles) and in Lake II (solid circles). The area under each curve is production as estimated by the Allen method. The broken line represents estimates for Lake II for 1982. Numbers at the arrows are numbers of larvae present on 31 May.

(95% C.L. of 225–3410) was similar to that recorded in 1982 (Fig. 2). The exponential model fitted to these two points was $N_t = 84\,882e^{-6.03t}$, where 84 882 larvae·ha^{-1} were estimated to be present on 31 May and $t = 1$ from 31 May to 30 September. The estimate of 85 000 larvae·ha^{-1} appears reasonable based on Wiedenheft's estimate of 60 adults·ha^{-1} on 26 May 1981 and a sex ratio of 1:1 for males and females (i.e. 30 females·ha^{-1} × 1754 eggs/female = 52 620 larvae·ha^{-1}).

Biomass and Production

In 1982 the maximum biomass of larvae of 48 kg·ha^{-1} in Lake I and 71 kg·ha^{-1} in Lake II occurred on 17 August (Table 3). From 31 May to 31 October the mean biomass in Lake I was 33.6 kg·ha^{-1} and in Lake II was 50.4 kg·ha^{-1}. Production for the same time period was 130 kg·ha^{-1} in Lake I and 146 kg·ha^{-1} in Lake II (Fig. 3). As the best precision for 95% C.L. of population estimates was ±20% of N, differences in biomass and production between the two lakes were not statistically significant.

In 1981, the maximum biomass of larvae recorded was 78 kg·ha^{-1} in Lake I on 16 September and 162 kg·ha^{-1} on 27 July in Lake II (Wiedenheft 1983). In Lake III the maximum recorded biomass of larvae was 182 kg·ha^{-1} on 8 August. Based on population estimates and mean weights of individuals on 27 July and 15 September and mean weight of individuals on 8 July, the annual production (31 May to 15 September) of larval salamanders was 565 kg·ha^{-1} in Lake II in 1981 (Fig. 4).

Discussion

In 1981 and 1982, the maximum biomass of salamanders occurred late in the growing season and was composed of larvae from the year's cohort. However, the seasonal trend of low biomass in the spring, composed of many small larvae, to high biomass in August–September, composed of relatively few large larvae, as recorded in 1982, may be altered considerably if there is good overwinter survival of larvae. In some years, large numbers of larvae overwinter and metamorphose in early spring to late summer and may contribute a significant biomass of large individuals in spring through summer. In spring and summer 1981, a large number (600–1000·ha^{-1}) of larvae overwintered in Lake II (Wiedenheft 1983). The biomass of salamanders of 153 kg·ha^{-1} in May (larvae comprised 91% of the population and their mean weight was 150 g; mean weight of adults was 85 g) nearly equalled the maximum biomass of 160 kg·ha^{-1} in late July composed of young-of-the-year larvae. Most larvae that overwintered metamorphosed and left the lake by late July.

The maximum annual production of larval salamanders was 565 kg·ha^{-1} for the 1981 cohort in Lake II. Lawler et al. (1974) reported a maximum annual yield of 313 kg·ha^{-1} for stocked rainbow trout in a Manitoba prairie lake; based on their annual mortality estimates of 50%, the maximum annual production would be nearly 350 kg·ha^{-1}.

High densities of larval salamanders in spring 1981 in Lake II apparently resulted in small-sized larvae by September–October (Fig. 3). The mean weight of larval salamanders in Lake II was 139 g in mid-September 1982, but was only 67 g in mid-September 1981. Densities of larval salamanders in Lake II in late July 1981 were about five times greater than in 1982; Wiedenheft estimated 5000 larvae·ha^{-1} (95% C.L. of 3860–7150) on 27 July 1981 compared with our estimates of about 1000 larvae·ha^{-1} in July 1982 (Table 3). The high densities of large larvae that overwintered of about 600–1000·ha^{-1} in Lake II

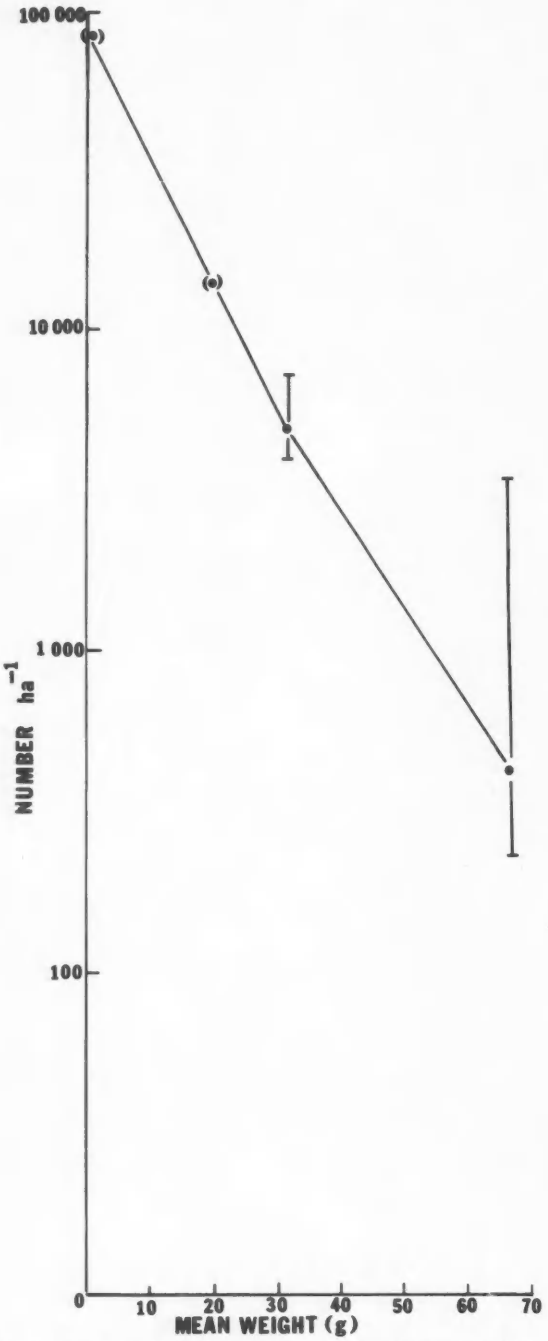

FIG. 4. Larval salamanders and their mean weights in Lake II in 1981. Parentheses indicate numbers of larvae that were estimated from the exponential model (see text). Vertical lines indicate 95% C.L. for *N*. Data from Wiedenheft (1983).

from May to June 1981 apparently had little influence on the growth of young-of-the-year larvae. The rate of growth of young-of-the-year larvae from early July to mid-September was linear and was 0.7 $g \cdot d_{-1}$. If competition with larvae that overwintered was important, we would have expected a change in the rate of growth of young-of-the-year larvae after July, as larvae that overwintered metamorphosed by late July and left the lake. Larvae were extremely small in Lake III in 1981 where there was a high density of both larval salamanders and fathead minnows (Wiedenheft 1983). Mean weight of larvae in Lake III was 59 g in late October 1981 and the estimated population of larval salamanders was 5280·ha^{-1} (95% C.L. of 3810–8570) on 8 August.

Larvae development was delayed at high densities. In 1981, no larval salamanders sampled on 15 September in Lake II had developed secondary sexual characteristics whereas in 1982, all larval salamanders had developed secondary sexual characteristics when sampled on 18 September.

The highest densities of larval salamanders of about 5000·ha^{-1} were recorded in 1981 by Wiedenheft (1983), when there were also high densities of larvae that overwintered (Lake II) and high densities of fathead minnows (Lake III), both potential competitors. Extensive food-habit studies of Myers (1973) and Deutschman (1984) recorded no larval salamanders in the diets of larval or adult salamanders. Amphipods, which occurred in extremely high densities in the lakes, were the principal organism eaten and may have accounted for the lack of cannibalism. The small mouths of fathead minnows make predation on larval salamanders unlikely. In four North Dakota lakes containing salamanders, Held and Peterka (1974) found that no salamander eggs or larvae were consumed by fathead minnows. Perhaps salamander larvae that overwinter and/or fathead minnows are important in reducing invertebrate predation on young-of-the-year larval salamanders. Potential invertebrate predators present in the lakes included odonate naiads, notonectids, and dytiscids. Holomuzki (1986) reported that predaceous diving beetles (*Dytiscus*) were important predators of larval tiger salamanders in Arizona.

We were unable to identify causes of mortality of salamanders other than observations of predacious birds (herring gulls, white pelicans, double crested cormorants, night herons, and great blue herons) on the lakes. Attacks by birds were probably responsible for a high incidence of wounds on larval salamanders (Weidenheft 1983). More than 10% of the salamanders captured in early August 1981 in Lakes I and II had wounds on the dorsal surface of their tails (Wiedenheft 1983); in 1982 the incidence of these wounds was about 2% in both lakes from August to late October (Deutschman 1984).

In spite of the large differences in densities of larvae in the spring of 1981 and 1982 in Lake II, by the end of the growing season, densities were similar at about 400 larvae·ha^{-1} (Fig. 2). This may indicate spacing behavior. Size-dependent spacing behavior was reported for larval and neotenic *Ambystoma* (Taylor 1983) in lakes in Oregon.

Success of salamanders in prairie lakes, where winterkills of larvae are common, hinges on the overwinter survival of adults which migrate to lakes and reproduce in the spring. Wilbur (1984) described amphibians' complex life cycles as adaptions to exploit transient opportunities for growth or dispersal.

In some populations, tiger salamanders develop cannibalistic morphs (Collins and Holomuzki 1984; Lannoo and Bachmann 1984) and are neotenic (reproduction by larvae) (Sexton and Bizer 1978). No evidence of cannibalism has been recorded for tiger salamanders in prairie lakes in this region (Dobie 1962; Olenick and Gee 1981; Myers 1973; Deutschman 1984). Although we were unable to resolve whether or not neoteny

occurs, it is unlikely, as we never observed this in the field. It is likely that some larvae that overwinter metamorphose into adults early in the spring before reproducing; we transferred some larvae we caught in the spring to the laboratory and they quickly (5–6 d) metamorphosed.

The accuracy of our population estimates relies on methodology developed by Myers (1973) and Wiedenheft (1983) in their attempts to estimate salamander densities in the lakes we studied. Their results, as well as ours, indicated that removal of phalanges did not cause mortality, at least when salamanders were held in live boxes in the field, and that marks were easy to observe. Wiedenheft (1983) used chi-square goodness-of-fit tests to determine if observed proportions of recaptured salamanders differed from expected proportions of salamanders marked at five permanent stations in each lake; salamanders were given unique marks at each station. His results indicated that in most cases, marked salamanders dispersed randomly with unmarked ones. Recruitment of small larvae that grew into the marked size range was only a problem in an 8 July estimate in Lake II in 1982 (Deutschman 1984); this estimate was not included in the data set. It is also likely that larvae that overwinter may metamorphose and this recruitment would cause an overestimate of the adult population. This may have occurred in 1981 in Lake II, but was not a problem in 1982 when no larvae overwintered. The male to female sex ratio of 1:1 for adults in Lake II in spring 1981 may have been influenced by the recruitment of metamorphosed larvae whose sex ratios in the spring were 1:3 (Wiedenheft 1983).

Our results have several implications for the aquaculture of fishes in prairie lakes in the prairie pothole region. They (1) may provide maximum potential values for secondary production, (2) suggest that there may be competitive interactions between salamanders and fishes, and (3) indicate that salamanders themselves may be important sources of protein.

Acknowledgements

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Focal Scale Damage Among Chum Salmon (*Oncorhynchus keta*) of Hokkaido, Japan

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Bigler, B. 1988. Focal scale damage among chum salmon (*Oncorhynchus keta*) of Hokkaido, Japan. Can. J. Fish. Aquat. Sci. 45: 698–704.

Fifty percent of chum salmon (*Oncorhynchus keta*) sampled from seven hatcheries in Hokkaido, Japan, were found to have either damage to the osseous scale layer where a hole resulted or intrusion of the osseous layer into the underlying fibrillary plate. Damage resulting in a hole is not related to age, body size, sex, or hatchery stock size. Intrusion of the osseous layer is similarly not related to body size, sex, or stock size; however, this phenomenon may be related to age. All damage was confined to the first, and rarely extended into the second, annual growth zone, suggesting occurrence during that period and retention of the mark throughout the life cycle. No evidence was found to support the hypothesis that either type of focal scale damage is a result of reduced diet. Examination of historical records from one hatchery revealed that the phenomenon of a hole has occurred for at least the past 10 yr at that location. It is not presently possible to identify hatchery of origin using these traits; however, such permanent, uniformly occurring traits may be of use in the identification of chum salmon originating from Hokkaido.

Chez 50 % des saumons kétas (*Oncorhynchus keta*) échantillonnés à sept piscifactures d'Hokkaido (Japon), on a noté des lésions de la couche écailleuse osseuse (présence de trous) ou une intrusion de la couche osseuse dans la plaque fibrillaire sous-jacente. L'incidence de lésions n'est pas liée à l'âge, à la longueur, au sexe ou aux effectifs gardés en piscifacture. De même, l'intrusion de la couche osseuse n'est pas liée à la longueur, au sexe ou aux effectifs gardés en piscifacture; toutefois, elle peut être liée à l'âge. Toutes les lésions étaient restreintes à la première zone de croissance annuelle et étaient rarement présentes dans la seconde zone; ceci porte à croire qu'elles apparaissent pendant cette période et qu'elles sont retenues pendant tout le cycle vital. On n'a relevé aucune preuve venant étayer l'hypothèse que les deux types de dommages localisés de la couche écailleuse sont le résultat d'un régime alimentaire inadéquat. L'examen de données historiques obtenues d'une piscifacture a révélé que la présence d'un trou a été observée pendant au moins les dix dernières années à cet endroit. Il est actuellement impossible d'identifier la piscifacture d'origine à l'aide de ces caractéristiques; toutefois, de telles caractéristiques permanentes uniformément présentes peuvent servir à l'identification du saumon kéta provenant d'Hokkaido.

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This paper presents the initial physical description and pertinent data analysis of focal scale damage resulting in a hole, or intrusion of the osseous scale layer into the underlying fibrillary plate, found among chum salmon (*Oncorhynchus keta*) originating from several hatcheries of Hokkaido, Japan (Fig. 1). The potential use of these traits for stock identification is also discussed. An explanation of these newly discovered scale traits is beyond the scope and intent of this paper. The author has been unable to locate any published reports concerning these focal traits among salmon. Furthermore, no reports have been found which discuss scale damage among any species where all materials are removed and a hole resulted, as this report will describe.

The cycloid scales of chum salmon initially develop when the fish attains a body length of approximately 3.8 cm (Yamada 1971). From the first rudiments of bony plates embedded in pockets of fibrous connective tissue, scales advance to become composed of a layer of calcified material and an underlying fibrillary plate which is composed of collagen fibrils. Growth resulting from cellular activity at the scale margin continues throughout life.

It can be assumed that the progression of scale resorption in chum salmon resembles that of Atlantic salmon (*Salmo salar*), as described by Crichton (1935). For that species, scale resorption commences when the fish stops feeding at the onset of the spawning migration. Following the cessation of feeding, cells which had previously functioned to enlarge the scale reverse function and commence removal of the ossified layer. As noted in Masu salmon parr (*Oncorhynchus masou*) by Ouchi et al. (1972), salmonid scale resorption generally starts at the posterolateral margin (that portion which protrudes from the skin surface) and continues around the scale. The fibrillary plate is eroded by mechanical or cellular action (J. Yamada, Faculty of Fisheries, Hokkaido University, 3-1-1, Minato-cho, Hakodate

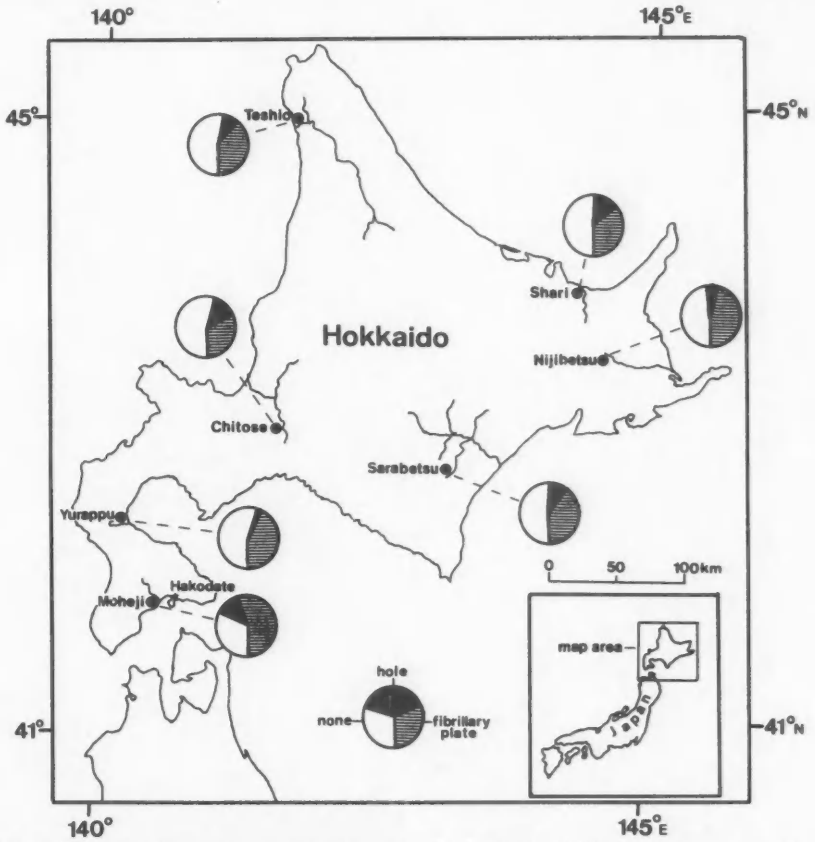

FIG. 1. Hokkaido, Japan, chum salmon hatchery collection sites, 1986. Pie charts represent the percentage of each focal scale damage type found.

041, Japan, pers. comm.) Crichton (1935) mentioned resorption on the Atlantic salmon scale "surface" in addition to that of the margin, but offered no elaboration.

Several authors have noted that focal scale damage (resorption) has resulted following experimentally induced periods of starvation in carp (*Cyprinus carpio*) (Ichikawa 1953) and goldfish (*Carassius auratus*) (Yamada 1956; Ikeda et al. 1974). A conclusion common among these authors is that resorption of material from the scale focus, as well as from the margin, represents the extreme and occurred under conditions of reduced diet.

Materials and Methods

Sample Collection and Handling

The phenomena described here were noted among scales collected from seven chum salmon hatcheries in Hokkaido (Fig. 1). The study of focal scale damage was not the original intent of this sampling effort. Fish were selected from among those captured as brood stock; however, samples of both sexes were not always possible due to special handling which occurred at each collection site.

Several scales were collected from the area on the left side just below the dorsal fin and two or three rows above the lateral line of approximately 250 fish from each location. These scales were either scraped from the skin using a knife, or a small area of skin was removed when scales could not be easily extracted. Scale samples were placed into envelopes, transported to the Hakodate campus of Hokkaido University, and frozen until processed. Fork length (mideye to tail fork) and sex were noted on each envelope.

Samples were thawed, removed from envelopes, momentarily dipped in tepid water, and gently rubbed with the fingers to remove adhering debris and tissues. Scales found to have mechanical damage were discarded. Six to eight scales from each sample were mounted between glass microscope slides and examined under 100× magnification for age determination. Age is recorded in European notation where two numerals which precede and follow a decimal point, respectively, indicate the observed number of freshwater and ocean annuli on the scale. It was during the process of aging that, besides normal resorption of the margin, scales from each location were noted to include varying degrees of focal damage involving the osseous and fibrillary plate scale layers.

Focal scale damage resulting in a hole (Fig. 2) is treated as a separate phenomenon from damage resulting from intrusion of the osseous layer into the fibrillary plate (Fig. 3). A hole is assumed to have resulted from forces exerted on the ossified layer and extending through the fibrillary plate.

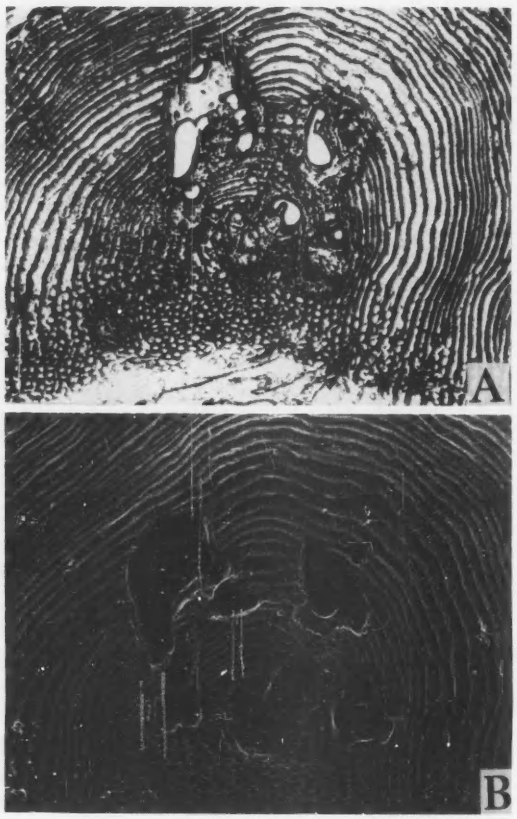

FIG. 2. (A) Light and (B) scanning electron micrographs of a scale sample representative of damage resulting in a hole. Magnification ×42.

Fibrillary plate damage, which was occasionally difficult to distinguish because of optical interference and a tendency to focus on annuli, was easily detectable by moving the examining projector focal plane through the scales. Where damage was unclear due to visual distortion, placing a few drops of water on the edge of the slides and allowing the water to fill all voids by capillary action made even very small damaged areas easily visible.

Thin Sectioning

Samples collected and placed in paper envelopes are not suitable for examination by thin sectioning. Several additional samples (not including the surrounding skin tissue) were collected, fixed in 1% glutaraldehyde for at least 4 h, washed with a 0.1 M phosphate buffer which had been adjusted to pH 7.4, dehydrated through an alcohol series to 100%, and embedded in epoxy resin. Sections of 0.5–1.5 μm were cut using a glass knife, mounted on microscope slides, and stained with silver nitrate.

Light and Scanning Electron Microscopy

Samples representing both types of damage were selected for light and scanning electron microscopic (SEM) examinations and photography. SEM examination allowed inspection of surface textures which could not be easily seen under light microscopy. All SEM samples were rapidly dehydrated through an alcohol series to 100% and mounted on brass specimen pegs to which a piece of double-sided tape had been applied. Mounted preparations were coated for approximately 3 min with gold–palladium in an ion sputter.

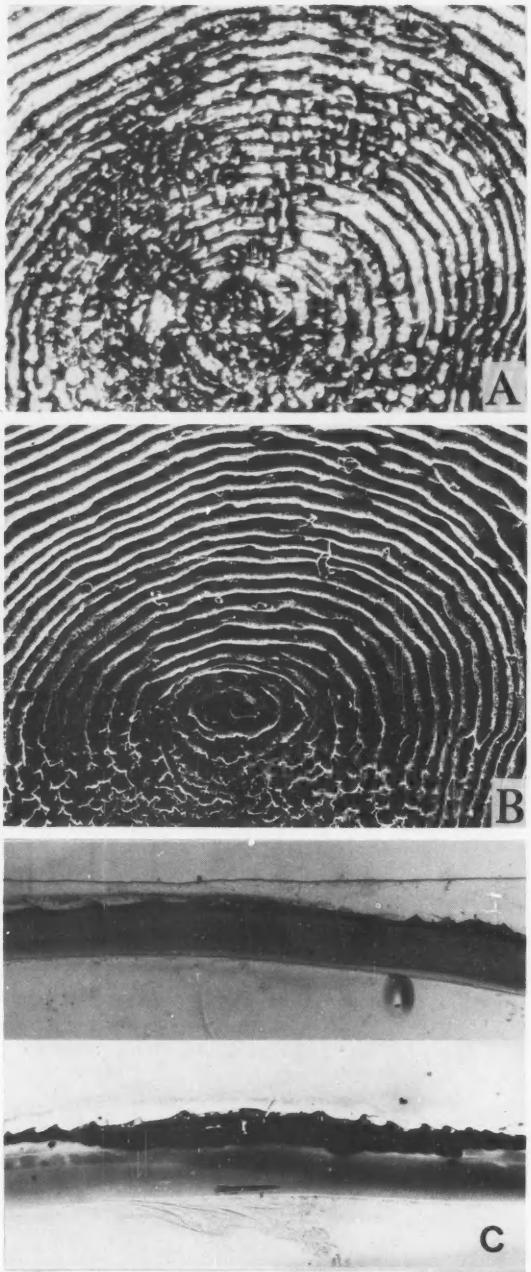

FIG. 3. (A) Light and (B) scanning electron micrographs of a scale representative of fibrillary plate damage. Magnification ×65. (C) Thin section of a normal scale (top) and a scale having intrusion of the osseous layer into the fibrillary plate (bottom). Broken areas of the osseous layer are artifacts of sectioning. Magnification ×200.

Examination of Historical Records

Historic scale records are invariably useful only for the study of damage resulting in a hole. With the advent of mounting scales on gum cards and pressing into acetate plastic (Clutter and Whitesel 1956), few scale collections are available in which both of these traits can be readily examined. Scale samples taken over the past 10 yr were obtained from the Moheji River Hatchery, located in southern Hokkaido (Fig. 1). These samples consisted of a single scale (taken from the same area of the body as is described above) from each fish, mounted on gum cards, and impressions made in acetate plastic.

Statistical Analysis

The frequency distribution of focal scale damage among age classes and sex was compared using a log-likelihood ratio test (Zar 1984). This test is most appropriate because of low expected values in some cases which place doubt on the interpretation of chi-square. Frequency distributions among observed fork lengths and circuli counts were tested using the nonparametric Kruskal–Wallis test with tied ranks. To simplify calculations, fork lengths were rounded to the nearest centimetre. All test results were judged at the $p = 0.05$ probability level.

Results

Not all scales from those fish classified as having focal damage showed such evidence. Also, because samples were taken from only one part of the body, only limited conclusions are possible concerning the frequency of these traits among the scales of an individual fish. In some instances, only one scale of those mounted was found with damage. At the opposite extreme, some samples were not usable for circuli counts because no complete scales were found.

Ossified Layer Damage

The extent of damage to the ossified scale layer ranged from small pits to the entire central region being resorbed resulting in a hole easily visible to the unaided eye (Fig. 2). Holes were usually surrounded by an irregular pattern of osseous layer distortions (Fig. 2A) and smooth areas where the ossified layer had been removed, exposing the underlying fibrillary plate (Fig. 2B). In some samples, a pattern of small circuli within the hole was noted, indicating the formation of reparative scale material (Fig. 4).

Fibrillary Plate Damage

Fibrillary plate damage most often appeared as an irregular, more or less radial pattern surrounding the scale focus when viewed with light microscopy (Fig. 3A). The visual distortion caused by this irregular pattern prevents further description. When viewed with SEM, scales which were found to have damage to the fibrillary plate had no evidence of damage to the ossified layer surface (Fig. 3B). Areas which appeared as an irregular pattern under light microscopy were found to be caused by intrusion of the ossified scale layer into the fibrillary plate (Fig. 3C).

Geographic Distribution

Combined focal scale damage traits were found in an average of 50.0% (SD = 8.8%) among each sampled population (Fig. 1). Among the hatcheries sampled, scale damage resulting in a hole ranged from 2.7% (Yurappu) to 15.3% (Chitose). Damage confined to the fibrillary plate was detectable among 29.0% (Chitose) to 55.0% (Moheji) of the fish sampled.

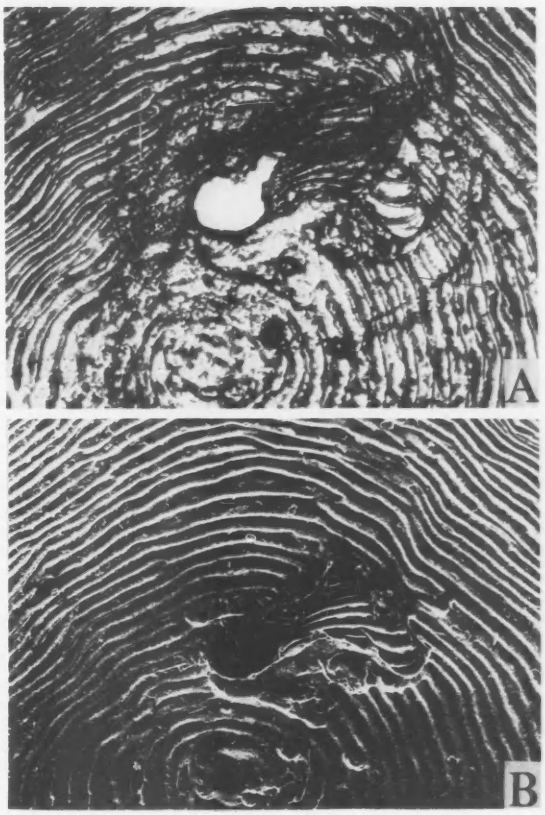

FIG. 4. (A) Light and (B) scanning electron micrograph of a scale sample in which repair to the damaged ossified layer is evident. Magnification × 65.

Analysis of Growth-Related Parameters

Results of tests to compare circuli counts between fish which possess either damage type and those which do not contradict the hypothesis that focal scale damage is a consequence of reduced diet. Several previous studies have found that changes in feeding level highly influenced circuli patterns (Grey and Setna 1931; Major and Craddock 1962; Bilton and Robins 1971a, 1971b, 1971c; Suzuki and Wada 1985). Assuming that focal scale damage is not limited to the spawning season, circuli counts within the first, second, and third year zones of age 0.3 chum salmon were compared. These comparisons revealed that in 18 of 21 cases, there were no significant differences in the numbers of circuli (Fig. 5). Where differences were detected, a multiple comparison indicated that scales in which focal damage was evident had significantly more circuli than those which did not.

Relation with Age

A significant correlation ($n = 0.941$) was found between age and the pooled percentage of fish with fibrillary plate damage.

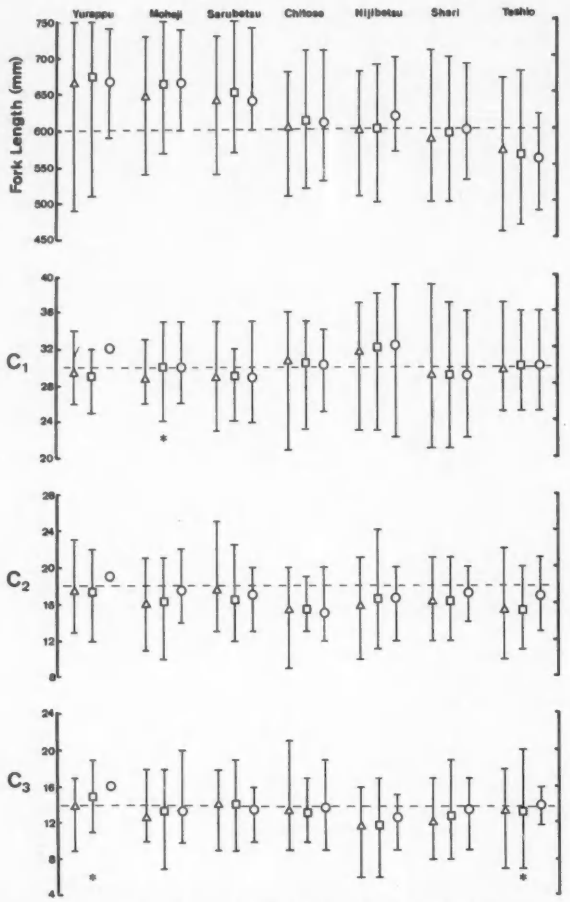

FIG. 5. Comparison of population forklengths and scale circuli numbers within the first (C_1), second (C_2), and third (C_3) year zones of age 0.3 chum salmon sampled from seven Hokkaido, Japan, hatcheries, 1986. Triangles, squares, and circles represent samples where no focal scale damage was found, damage to the fibrillary plate, and damage resulting in a hole, respectively. An asterisk indicates where scales found with either focal scale damage type had significantly more circuli than those which had no damage.

This correlation implies that the incidence of this trait increases with time at sea. A correlation of $r = 0.338$ indicated no relationship between age and the percentage of damage resulting in a hole. No significant differences were found in the frequency distribution of damage types among age classes within each hatchery stock, but significant between-population differences were detected such that these data cannot be combined to one set (Table 1). On the other hand, if these data were taken prior to capture at the hatchery sites, the origin would have been unknown. Assuming no knowledge of origin, the data were pooled by age class and the percentage of fish possessing each focal scale damage type was correlated with age. The significant correlation between age and the pooled percentage of fish with fibrillary plate damage lacks support from the test which compared damage frequency with body size, also an age-related variable.

TABLE 1. Summary of log-likelihood ratio test for homogeneity of focal scale damage among age classes and sexes of chum salmon collected from seven hatcheries in Hokkaido, Japan, 1986.

Hatchery location	Tested variable	n	G[a]
Moheji	Age	223	3.285
Chitose	Age	249	9.105
Teshio	Age	278	4.281
Shari	Age	214	3.857
Yurappu	Age	263	7.145
	Sex	263	0.146
Nijibetsu	Age	245	2.736
	Sex	245	3.666
Sarubetsu	Age	243	6.166
	Sex	243	1.622
Combined	Age	1714	25.474[b]
	Sex	751	4.370

[a]Calculated according to Zar (1984).

[b]Exceeds the appropriate critical value (15.507). Conclusion: the three classifications of focal scale damge (i.e. none, fibrillary plate, or a hole) are not uniform between hatchery age classes.

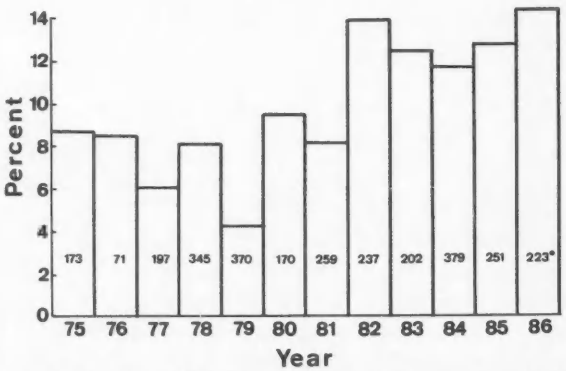

FIG. 6. Percentage of Moheji hatchery brood stock found to have focal scale damage resulting in a hole, 1975–86. Sample sizes are noted within the bar graph for each year. Data from 1975–85 are based on a single scale impression in acetate plastic. Data from 1986 (asterisk) are based on six or more scales mounted between glass slides.

Other Tests

Both classifications of damage were found uniformly distributed among all other variables tested. Focal scale damage was concluded to be uniformly distributed among the various body sizes found within hatchery populations (Fig. 5). Similarly, no differences were found in the frequency distribution of these damage traits between sexes, within and between hatchery populations (Table 1). The incidence of damage was also not correlated with the number of releases from each hatchery ($r = 0.09$ and $r = 0.17$ among age 0.3 fish with damage resulting in a hole and that of the fibrillary plate, respectively; data source: Hokkaido Regional Salmon Hatcheries 1983).

Historic Data

Scale damage resulting in a hole within the Moheji hatchery brood stock has occurred for at least the past 10 yr, and has

ranged from 4.3% in 1979 to 13.9% in 1982 (Fig. 6). It was not possible to assess fibrillary plate damage because these data are taken from plastic impressions. Nor is it possible to discuss any apparent tendencies for the incidence of this trait to change over this period. Such a discussion would necessarily assume sampling bias, in the form of discarding obviously damaged scales or the selection of a single scale from each fish, to be constant or negligible, assumptions which cannot be guaranteed. However, in spite of the disparity between sample sizes from each fish, the value of focal scale damage resulting in a hole among this hatchery stock in 1986 (14.3%) compares closely with values of other recent seasons (asterisk in Fig. 6).

Conclusions

Among the data collected from Hokkaido chum salmon in 1986, no physical or population parameter was found that clearly differentiates fish possessing either focal scale damage type from those which have no damage. Furthermore, no test among growth-related variables (body length, circuli numbers) supports the hypothesis that focal scale damage as described here results from a reduced diet, as has been noted in other species.

The significant relationship between fibrillary plate damage and pooled age classes lacks supportive results from other tests, and interpretation of this is not straightforward. The relationship suggests that the incidence of fibrillary plate damage is related to the time spent at sea. If this is a true relationship, corroborating evidence should have been found in the frequency distribution of this trait among the various body sizes. Although not completely dismissible, the lack of tendency for more larger (older) fish to possess this trait places doubt on the correlation with pooled age data.

Historic data in the form of acetate impressions provide a valuable data source for detecting focal scale damage resulting in a hole. In past sampling efforts, however, collection of one scale per fish, coupled with the potential tendency for technicians to discard what appear to be damaged scales, would likely result in underestimating the incidence of this phenomenon. Among samples collected from Moheji hatchery in 1986 (Fig. 1), where the collection of skin patches resulted in a relatively larger number of scales per fish available for examination, 72% of the scales taken from fish classified as possessing focal damage resulting in a hole had a hole (n = 36; SD = 25.4%; mean sample size per fish = 14.4; range = 4–34). This indicates that up to 28% of the fish from this hatchery designated as having a hole may not have been detected if only one scale had been collected. This bias would have been compounded if technicians had tended to sort out what appeared to be damaged scales. The possibility of detecting focal scale damage, therefore, increases with sample size from each fish. Comparisons of data based on the sampling of one scale and those of larger sample sizes should be performed with this in mind.

These phenomena were first noted among Moheji hatchery samples and it is reasonable to suggest that samples processed prior to that time (Teshio, Chitose, and Shari) were subjected to a certain amount of scrutiny in which obviously damaged scales were discarded. Such bias would reduce the frequency of either trait noted among samples, but judging from the remarkable uniformity at which these combined traits occur among most hatchery sites this bias is most likely not significant.

With further study, these scale traits may prove valuable for stock identification. Confinement of damage to the scale focus, as well as evidence that reparative scale material had been laid down, indicates that these traits developed prior to the spawning season and most likely during the first year of life. In the case of reparative scale material, it is unlikely that normal resorption of the scale margin during the spawning season (when these samples were collected) would coincide with reparations to the focus. Thin sections of scales where fibrillary plate damage was noted did not include any structures that would indicate an active process. This suggests that damage was initiated during the first, and occasionally extending into the second, year of life when these materials were initially laid down and remains as a permanent mark. That these phenomena occur to such a uniform degree suggests that Hokkaido chum salmon are influenced as a population and differences found between hatcheries may be attributable to sample size or an expression of differing tendencies to develop either trait. No attempt was made in this study to compare the physical appearance of either trait between hatcheries, and no qualities of these phenomena can be readily identified which would allow identification of a particular fish to a particular hatchery. It is obvious, however, that if these traits are unique to Hokkaido chum salmon, or if the incidence of these traits among other populations is sufficiently distinct, such unique, uniformly occurring, easily identifiable scale characters will provide a valuable tool for stock identification. It is desirable to know whether these traits are also common to other North Pacific populations of chum salmon.

Acknowledgments

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Effect of Black Fly Ingestion and Assimilation on Seston Transport in a Quebec Lake Outlet[1]

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Ingestion and assimilation rates of simullid (Diptera) larvae feeding on natural seston were estimated in an artificial stream and used to predict the impact of simuliids on seston transport in a 40-m section of the outlet stream of Petit Lac Fraser in southern Quebec. This prediction was then compared with changes in the seston flux above and below the population. Finally, we selectively killed simuliids with a larvicide (*Bacillus thuringiensis* serovar. *israelensis*) to observe changes in seston transport after removal of simuliids. The stream population of simuliid larvae ingested between 32 and 55% of the seston flux and assimilated between 17 and 25% of the dry mass of ingested seston. The change in assimilation of seston in the stream section before and after the larvicide treatment was similar to that predicted from assimilation rate of larvae and standing stock. Larvae ingested 0.8–1.4% of the seston per linear meter of stream, about 10–1000 times more than what has been reported for other populations of stream filter-feeders. The high values observed can be explained by high specific ingestion rates, high standing stock, and low discharge. Our results suggest that simuliid populations can be food limited and may help to explain the downstream decrease in abundance and growth rates of filter-feeders in lake outlets.

On a déterminé les taux d'ingestion et d'assimilation chez des larves de Simullidae (Diptères) qui avaient consommé du seston naturel dans un cours d'eau artificiel; ces taux ont servi à la prédiction de l'incidence des Simullidae sur le transport du seston dans un tronçon de 40 m de l'émissaire du Petit lac Fraser (sud du Québec). On a ensuite comparé cette prédiction aux variations du seston en amont et en aval de la population de Simullidae. En dernier lieu, on a sélectivement éliminé ces dernières à l'aide d'un larvicide (*Bacillus thuringiensis* serovar. *israelensis*) afin d'observer les variations du transport du seston après la disparition des Simullidae. La population fluviatile de larves de Simullidae a consommé de 32 à 55 % du seston et a assimilé de 17 à 25 % de la masse sèche du seston consommé. La variation du taux d'assimilation du seston dans le tronçon du cours d'eau avant et après l'application du larvicide était semblable à celle prédite d'après le taux d'assimilation des larves et le stock actuel. Les larves ont consommé de 0,8 à 1,4 % du seston par mètre linéaire de cours d'eau, soit de 10 à 1 000 fois plus que les taux signalés chez d'autres populations de filtreurs fluviatiles. Les taux spécifiques d'ingestion et le stock actuel élevés, ainsi que le faible débit peuvent expliquer les valeurs élevées observées. Les résultats portent à croire que les populations de Simullidae peuvent être limitées par la quantité de nourriture; ils peuvent aussi expliquer la baisse de l'abondance et des taux de croissance des filtreurs dans les eaux d'aval à l'émissaire des lacs.

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Large populations of filter-feeding black fly larvae are commonly found in the outflows of lakes, dams, and reservoirs (Hynes 1970). Their abundance in outlet streams is positively related to chlorophyll concentration, protein concentration, or organic carbon concentration in the water (Gíslason 1985; Morin and Peters 1988). Within outlet systems, density and growth rate of the larvae are maximum immediately below the lake and decline downstream (Lewis and Bennett 1975; Carlsson et al. 1977; Sheldon and Oswood 1977; Back and Harper 1979). This decline in abundance and productivity of larvae has been related to the gradual disppearance of the planktonic particles coming from the lake (Reif 1939; Maciolek and Tunzi 1967; Hynes 1970; Carlsson et al. 1977; Wotton 1979). Food therefore seems a probable factor limiting black fly abundance. However, the mechanisms underlying the downstream decrease of black flies and of their food are still unclear.

[1]Contribution of the Limnology Research Centre of McGill University and the Groupe de Recherches sur les Insectes Piqueurs of Université du Québec à Trois-Rivières.

Physical and biological mechanisms could produce the downstream decline of lake plankton. Fragile algae and zooplankton can be destroyed by abrasion on the stream bottom or by high turbulence in the outflow (Maciolek and Tunzi 1967). Lake particles could also sediment or be captured by weed beds (Reif 1939). Finally, black flies and other filter-feeders may assimilate enough of the lake plankton to reduce its concentration for downstream populations (Sheldon and Oswood 1977). Unfortunately, the relative importance of these processes has not yet been quantified.

Estimates of the ingestion rate of seston by filter-feeders vary between 0.00008 and 0.019% of the organic seston per metre of stream bed (McCullough et al. 1979; Benke and Wallace 1980; Haefner and Wallace 1981; Ross and Wallace 1983; Parker and Voshell 1983). Since only a fraction of the ingested food is assimilated and the rest is released into the water, these estimates exaggerate the effect of filter-feeders on seston transport. Nevertheless, these values are so low that they make physical destruction and sedimentation appear the most likely explanation for the longitudinal decline of lake plankton in lake outlets. However, existing measurements of seston ingestion were obtained either in large rivers where the densities of filter-feeders were high or in small headwater streams where the filter-feeders were not abundant. There are no estimates of seston removal and assimilation for small lake outflows heavily colonized by filter-feeders. It is in these small systems, where the interface between flowing water and the substrate colonized by filter-feeders is extensive, that animals have the largest potential to alter seston quality or quantity and that the steepest longitudinal decline of filter-feeders has been observed (Morin and Harper 1986; Morin 1987).

The goal of this study was to quantify the impact of a large simulid population on the transport of seston in a small lake outlet. To this end, we first measured the ingestion and assimilation rates of black flies on natural seston in a seminatural system and then compared the estimated impact of black flies on seston with the observed changes in the seston passing over the stream population; finally, we described the changes in seston transport after the larvae had been selectively removed with a larvicide (*Bacillus thuringiensis* serovar. *israelensis*).

Materials and Methods

Study Site

The study was conducted in May 1986 at the outlet of Petit Lac Fraser, in the Réserve Saint Maurice, in the southern Laurentian region of Quebec (46°57′N, 73°02′W). Petit Lac Fraser is a small (0.5 km²), shallow lake (maximum depth = 3.5 m) in a watershed clearcut in the 1950's. The lake empties in a small cascade (1.9 m) and the stream then flows rapidly downstream (slope 2.5%), although current is reduced at the sides of the pool immediately below the cascade (Fig. 1). The studied section was the first 40 m of the outflow. The substrate is composed of cobbles and pebbles and is free of sediment and rooted plants in the main channel. The water depth varies from 5 to 25 cm in the main channel. Discharge in the outflow varied from 120 to 200 L/s in May when the study was performed.

At the time of the study, the stream population was composed mainly of larve of the *Simulium venustum/verecundum* complex, *Simulium tuberosum*, and *Simulium decorum*. A few larvae of the following species were also found but formed less than 0.5% of the total standing stock: *Prosimulium mixtum/fuscum*, *Stegopterna mutata*, *Cnephia dacotensis*, and *Simulium* spp. (probably *S. aureum* and *S. croxtoni*). Hydropsychid caddisflies (*Hydropsyche betteni* and *Cheumatopsyche pettiti*) and other invertebrates (mainly midges) were relatively rare in the section (50 and 1800 ind./m², respectively).

Description of the Gutter System

Ingestion and assimilation rates of larvae were measured in an artificial stream placed over the studied section. The system

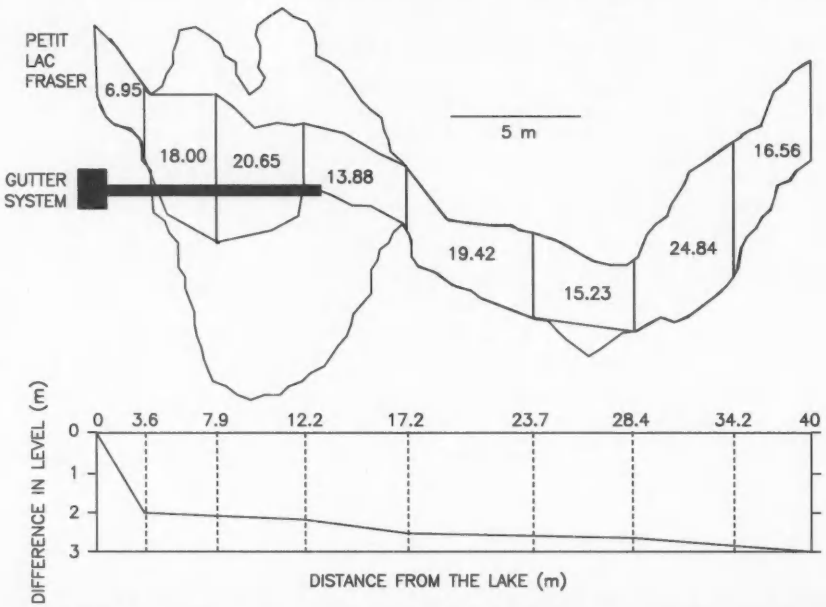

FIG. 1. Map and vertical profile of the study reach. The numbers indicate the planar surface area (m²) of each subsection. Longitudinal distances were measured along the centre of the channel.

consisted of a 25-L plastic sink, continuously filled with lake water brought through a pipe by gravity, and connected to three contiguous 2.4-m sections of U-shaped vinyl gutter (width = 10 cm). The sink was continuously overflowing, so the outflow in the bottom drain to the gutter remained stable at 0.045–0.05 L/s. Water velocity during the experiments was measured as the length of the gutter divided by the travel time of a slug of dye along the gutter. Because the larvae in the system greatly reduced the flow, the velocity was adjusted to about 40 cm/s, after the gutter was colonized with larvae, by varying the slope of the gutter.

Measurements

Discharge through the gutter was measured as the volume of water collected in 1 min. The variability among three replicates taken at the beginning of each experiment was always less than 2% of the mean. Discharge of the stream was estimated from a stage-level regression determined during a preliminary study in 1985. Estimates were compared on three occasions with discharge measurements obtained by measuring mean water velocity in the water column and water depth at 12–15 points across the stream. In all cases, estimates from the stage-level regression were within 5% of the measured values.

Concentration of seston was measured by filtering 200–250 mL of water on preweighed Nuclepore filters (0.2 μm pore diameter). Filters were kept frozen (−20°C) until return to the laboratory, where they were dried for 24 h at 60°C. After cooling in a desiccator, filters were reweighed to the nearest microgram on a Cahn electrobalance.

Standing stock in the gutter system was measured at the end of each experiment by gently scraping the gutter to dislodge the larvae. These were collected in a sieve (75 μm) placed in the gutter outflow and preserved in 4% formaldehyde. To describe the size distribution of larvae in the gutter, the preserved samples were subsampled with a Folsom plankton splitter to isolate about 500 larvae which were then measured to the nearest 0.1 mm. The rest of the larvae were weighed after drying for 24 h at 60°C. Mass was not corrected for losses due to preservative because no significant loss was detected after a similar preservation (1 wk) in a preliminary experiment (A. Morin, unpubl. data).

Standing stock in the stream was calculated from estimates of biomass per unit of rock surface area, estimates of rock surface area per planar area of suitable substrate, and measurement of the planar area of suitable substrate in the section. Twenty rocks (surface area 60–200 cm^2) were collected at random coordinates in the stream section and preserved in 95% ethanol. In the laboratory, rocks were gently scraped over a sieve (75 μm), and larvae were sorted, identified, and measured. Large samples were subsampled with a Folsom plankton splitter to yield 300–400 larvae in the subsample. We estimated the total surface area of each rock from the mass of aluminum foil necessary to wrap each rock (Morin 1987). The biomass of larvae on each rock was obtained by converting lengths to dry mass, using regression equations developed from fresh larvae. Rock surface area per planar area of stream was estimated from five replicate samples. We collected all rocks from the top layer in 0.25-m^2 quadrats and estimated the total surface area of these rocks from the mass of aluminum foil used to wrap them. The planar area available to simuliid larvae was estimated from the water surface area of the erosional part of the stream in the first 40 m. Because the stream flows over bedrock in the cascade immediately below the lake, larvae there were sampled with a mini-Hess sampler, similar to the T-sampler of Resh et al. (1984). Four replicate samples were collected in the cascade. Total surface area of bedrock was then measured. Benthos in the stream was sampled twice, just before the larvicide treatment on May 14 and 16 h after the treatment on May 15.

To estimate the density of the gut contents of larvae, we collected larvae in the streams, killed them in formaldehyde, and extracted the peritrophic membrane and the ingesta it contained within 2 h. We then measured the length and diameter of the contents of the midgut, removed the peritrophic membrane, and transferred the extract to a petri dish. We combined gut contents of 3–20 larvae of similar length (within 0.3 mm) for each measurement. The contents of the petri dish was then filtered onto preweighed 0.2-μm Nuclepore filters and dry mass estimated following the protocol for seston. Density of gut contents was calculated as the ratio of the dry mass collected on the filter to the total volume of extracted gut contents.

Ingestion rates of larvae were measured in the gutter system as the volume of gut filled per unit time (Ladle et al. 1972). After larvae were acclimated to the gutter (8–12 h), and after seston was measured there, we added a suspension of Norite (fine-grained charcoal) for 3 min at the gutter inlet, let the larvae feed for 10 min, and then added Norite again for 3 min. We collected a fraction of the larve, 5 min after the end of the last application of Norite, by scraping a narrow band of larvae along the gutter with a spatula. The larvae were then preserved in formaldehyde. In the laboratory, we measured the length (to the nearest 0.1 mm) and diameter (to the nearest 0.02 mm) of the cylindrical portion of the gut enclosed between the two Norite bands to estimate the volume ingested.

Assimilation rate of seston by larvae in the gutter system was calculated from the difference in flux of seston (grams of seston per minute) between the input and the output of the gutter. Twelve replicate samples were collected in the sink at the top of the gutter and at the outflow to measure seston and chlorophyll concentration. Specific assimilation rates of larvae in the gutter were calculated as

$$(1) \quad A = Q_g (C_u - C_d)/M_g$$

where A is the assimilation rate (micrograms dry mass (DM) of seston per milligram DM of larvae per minute), Q_g is the discharge in the gutter (litres per minute), C_u and C_d are the concentrations of seston upstream and downstream the gutter (micrograms per litre), and M_g is the mass of larvae in the gutter (milligrams DM). Control experiments in 1985 showed no difference in seston flux between the top and bottom of the gutter.

Experimental Design

Ingestion and assimilation rates were measured three times on May 13 and 14. The gutter was first colonized by placing stream rocks with their attached larvae into the gutter. Larvae were gently dislodged from the rocks so that they would attach to the gutter. Once the gutter was fully colonized (biomass equivalent to 30 g DM·m^{-2}), the rocks were removed and the larvae left to acclimate for 8–12 h. Water temperature in the gutter was always 0.5° higher than in the stream during daytime. After the acclimation period, we first collected water samples for the estimation of assimilation rate, then proceeded with the experiment to determine ingestion rate, and then collected larvae for the measurement of standing stock. After the experiment, the gutter was brushed clean before being recolonized for the next set of experiments (Fig. 2).

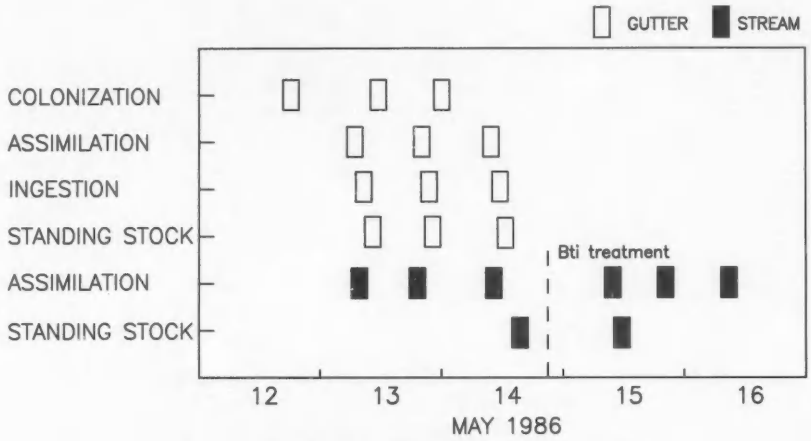

FIG. 2. Timing of the gutter and stream experiments.

TABLE 1. Length–mass regressions. Listed are the intercept (a), slope (b), residual mean square (RMS), coefficient of determination (R^2), and the number of data points (n) for the model $\ln W = a + b \ln L$ where W is dry mass (micrograms) and L is body length (millimetres).

Species	a (SE)	b (SE)	RMS	R^2	n
Simulium venustum/verecundum	0.553 (0.118)	3.036 (0.081)	0.043	0.98	38
Simulium decorum	1.192 (0.586)	2.692 (0.303)	0.027	0.89	15
Simulium tuberosum	0.247 (0.130)	3.342 (0.093)	0.047	0.95	70
S. venustum/verecundum and *S. decorum*	0.727 (0.086)	2.934 (0.054)	0.053	0.96	53

Water samples from the stream were collected by immersion of sample bottles at the mouth of the lake above the cascade and at 40 m below the lake. Twelve replicate samples were collected before the gutter experiments on May 13 and 14 and on three occasions after the larvicide treatment on May 15 and 16.

The larvicide treatment was applied at 2100 on May 14. Teknar HPD (500 mL) was applied at the mouth of the lake in 10 min. This corresponds to a dose of 3333 $mL \cdot s^{-1} \cdot L^{-1}$, about four times the dose that kills 95% of the largest larvae in bioassays performed in similar conditions (C. Back, unpubl. data). This insecticide is selective to black flies and some midges and does not induce drift or mortality of hydropsychids (Back et al. 1985; Gibbs et al. 1986).

Data Analysis

Length-mass regressions were fitted for *S. venustum/verecundum*, *S. decorum*, and *S. tuberosum* by ordinary least-squares:

$$(2) \quad \ln W = a + b \ln L$$

where L is the length (millimetres) of larvae killed in formaldehyde and immediately measured, W is the dry mass (micrograms), and a and b are the fitted intercept and slope. The regressions equations were back-transformed to arithmetic units and corrected for bias due to the logarithmic transformation

$$(3) \quad W = e^a L^b \, CF$$

where CF is the correction factor for the transformation (Sprugel 1983). It is equal to exp (0.5RMS) where RMS is the residual variance of around the log–log regression. Length–mass regressions for the three species are presented in Table 1. Analysis of covariance revealed that the regression equations for *S. venustum/verecundum* and *S. decorum* were not significantly different from each other but differed significantly from *S. tuberosum*. We therefore pooled *S. venustum/verecundum* and *S. decorum* data and used a single regression for these two species.

We also estimated regressions relating the density of the gut contents and the ingestion rate to body length using models similar to Eq. 2. Average specific ingestion rates for the gutter populations (I, micrograms DM of seston per milligram DM of larvae per minute) were calculated by combining the length – ingestion rate regression, the length – density of gut contents regression, and the size distribution of larvae in the gutter for each experiment. We first calculated the total mass of seston ingested per minute by each size class of larvae by multiplying the number of larvae in the size class, the volume ingested per larvae per minute, and the density of the gut contents of larvae in the size class. We then summed the results for all size classes and divided by the total mass of larvae in the gutter.

Total biomass in the stream section was calculated as the sum of the standing stock in each subsections illustrated in Fig. 1. Because density of the three dominant species declined about 10 times from the top to the bottom of the section (Fig. 3), we fitted a log-linear model to describe the relationship

$$(4) \quad \ln (N_{ij} + 100) = f + g \, (\text{distance from the lake (metres)})$$

where N_{ij} is the density of size class i of species j per rock surface area (individuals per square metre). Because some of the 0.1-mm size classes were absent from some samples, we added 100 (the detection limit for density per square metre on rocks of 100 cm^2) before the logarithmic transformation. To obtain the mean density for each k subsection (N_{ijk}, individuals per square metre), we divided the integral of the back-transformed equation, relative to distance from the lake (D, metres), by the length of the subsection (l_k, metres):

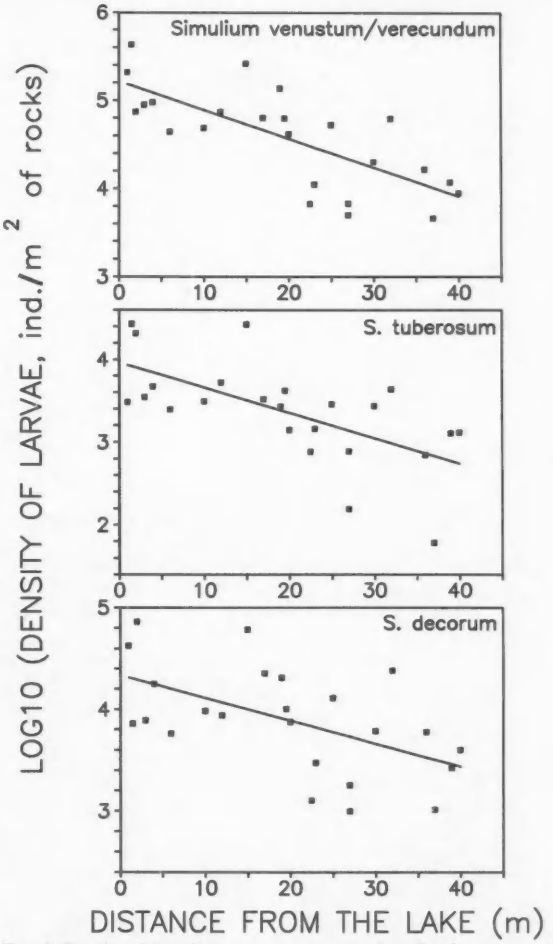

FIG. 3. Density of *Simulium venustum/verecundum*, *S. tuberosum*, and *S. decorum* against distance from the lake.

$$(5)\quad N_{ijk} = \frac{1}{l_k}\int_{D_{min}}^{D_{max}} [\exp(f + gD + 0.5\mathrm{RMS}) - 100]dD$$

$$(6)\quad N_{ijk} = \frac{\exp[f + 0.5\mathrm{RMS}]}{gl_k}[\exp[gD_{max}] - \exp[gD_{min}]] - 100$$

where D_{min} and D_{max} are the distances from the lake at the top and bottom of the subsection and 0.5RMS is the correction factor for the bias of the transformation.

The standing stock in the stream was calculated from the density of each size class of each species in each subsection (N_{ijk}, Eq. 5), the dry mass of larvae of size class i of species j, the surface area of the subsection (square metres), and the average ratio of rock surface area per planar area of stream (dimensionless, equal to 3.04 (SE = 0.47) for subsections 2–8 and assumed to be 1 in the cascade).

Error Analysis

Because computations involved various combinations of equations estimated on transformed data, of measured values, and of values prone to error but for which we did not have variance estimates, we could not combine errors to calculate parametric confidence intervals on most computed values. However, because estimates of uncertainty are necessary to compare statistically observed and predicted values, and to judge the quality of the results, we wrote several Monte-Carlo simulations (in TURBO BASIC®) to estimate approximate confidence intervals.

TABLE 2. Assumed coefficient of variations for measured values without error estimates.

Variable	Coefficient of variation (%)
Total mass in gutter	5
Discharge in gutter	2
Number of larvae in each size class in gutter	25[a]
Discharge in stream	5
Planar surface area in each subsection	10

[a]Estimated from 1/32 or 1/64 subsamples.

Most computations involved three components: regression equations, measured values with error estimates, and measured values without error estimates. For each computed value, we calculated an approximate 95% confidence interval in the following way: (1) we introduced sampling error in each component involved in the computation, (2) we calculated the desired value and the result was stored, (3) steps 1 and 2 were repeated 400 times, and (4) from the 400 resulting values, the limits of the 95% confidence interval were estimated as the 2.5 and 97.5 percentile of the distribution.

We introduced error in regression equations by randomly sampling, with replacement, the original data set. Then we recalculated the regression equation on the "bootstrapped" data set and used the equation for one run of the Monte-Carlo simulation. Error on measured values was introduced by adding a normal deviate to the measured value. The normal deviate had a mean of 0 and a standard deviation equal to the standard error of the mean of the measured value. For measurements without error estimates, we added a normal deviate with a coefficient of variation that seemed realistic (Table 2).

Several assumptions were made for the calculations, and therefore, some potential sources of error and bias were omitted from the error analysis. We assumed that ingestion rates in the gutter represented those in the stream, that larval ingestion was not affected by the two applications of Norite, and that neither the 0.5° difference between the water in the gutter and the stream nor the slight decrease in seston concentration between the top and bottom of the gutter affected ingestion rates. We also assumed that the assimilation efficiency did not vary among species or size classes. Finally, we assumed that no significant changes in mean individual mass and standing stock occurred in the stream over the 36 h of experiments before the larvicide treatment. Most of these assumptions are plausible but were not tested, and therefore, confidence intervals can only be approximate.

Results and Discussion

Density of Gut Contents

Density of the gut contents of the three species declined exponentially with increasing body length (Fig. 4), resulting in a fourfold difference between the smallest and largest larvae.

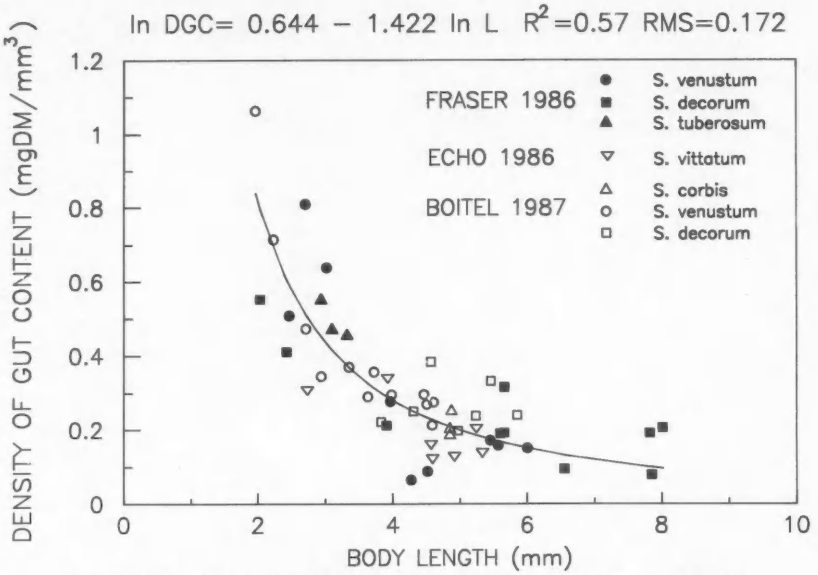

FIG. 4. Density of gut contents (DGC) of simuliid larvae against their body length (*L*).

No significant differences were detected among species, and the data from the outlet of Petit Lac Fraser were similar to those obtained in two other lake outlets from the southern Quebec. We therefore used both data sets to fit a single regression model. The pooled regression was not significantly different from the regression on Petit Lac Fraser data only (ANCOVA, $P > 0.5$), and the inclusion of the additional data points decreased the standard errors on both the slope and intercept of the regression.

Differences in density of the gut contents of small and large larvae may reflect particle size selectivity. If small larvae ingest proportionally more small particles than large larvae, their gut contents could be more densely packed. However, there is conflicting evidence on the particle size selectivity of larvae of different sizes (Schröder 1981), and it is unclear whether the relatively slight differences in the size spectrum utilized by larvae of different sizes could produce the difference we observed between the smallest and largest larvae.

Ingestion and Assimilation Rates

Length – ingestion rate regressions for the three species are presented in Table 3. Both *S. venustum/verecundum* and *S. tuberosum* ingestion rates increased significantly (ANCOVA) from the first ingestion experiment (May 13, a.m.) to the last experiment (May 14, noon), as did water temperature (from 14 to 18°C) and average individual mass (from 77 to 123 μg). We therefore fitted equations for each period for these two species. For *S. decorum* we had fewer observations and could not detect significant differences in ingestion rate among the three experiments; we therefore fitted a single equation for this species.

Results of the experiments for estimating the assimilation rate of larvae are presented in Tables 4 and 5. Specific assimilation rates increased from 0.19 to 0.39 $\mu g \cdot mg\ DM^{-1} \cdot min^{-1}$ between the first and third experiment. Assimilation efficiency on natural seston did not vary significantly among the three experiments and averaged 22.5%.

Using labelled cultures of diatoms, McCullough et al. (1979) measured specific ingestion rates varying between 0.2 and 1.3 $\mu g \cdot mg\ DM^{-1} \cdot min^{-1}$ for late instar larvae of simuliids. Wotton (1978) reported specific ingestion rates ranging from 10.8 to 14.0 $\mu g \cdot mg\ C^{-1} \cdot min^{-1}$. Our results for ingestion rates, averaging 1.2 $\mu g \cdot mg\ DM^{-1} \cdot min^{-1}$, therefore lie within published values.

Schröder (1981) collated literature values of assimilation efficiency of simuliids and reported efficiencies ranging from 1.8 to 79% depending on the food particle type. Our assimilation efficiencies of about 25% are below most results with cultured diatoms and green algae, but are above the assimilation efficiency on detritus reported by Wotton (1978).

Stream Experiment

The 40-m stream section harbored about 35 million simuliid larvae before the insecticide treatment (Table 4). The size distribution of the three species differed (Fig. 5). Both *S. venustum/verecundum* and *S. decorum* had a bimodal distribution; but most larvae of *S. venustum/verecundum* were smaller than 4 mm whereas the majority of *S. decorum* larvae were larger than 4 mm. *Simulium tuberosum* had a unimodal distribution, with most larvae between 3 and 5 mm. After the insecticide treatment, we found only 21 first instar larvae and 2 parasitized last instar larvae of *P. mixtum/fuscum* in the 24 benthos samples. We thus achieved more than 99.999% mortality of the larvae present before the treatment and assumed that biomass of simuliids in the stream was null after the treatment. We did not detect any significant changes in densities of hydropsychids or other invertebrates.

When black fly larvae were present, between 8 and 9% of the seston flux was assimilated between the top and bottom of the section (Table 6). This relative assimilation did not differ significantly from what was predicted from the standing stock and specific assimilation rate of the larvae (from 6 to 14%). However, confidence intervals on the relative assimilation in the section ranged from negative to about 20%, and therefore, no significant loss of seston between the top and bottom of the

TABLE 3. Length – ingestion rate regressions for the three species. Listed are the intercept (a), slope (b), residual mean square (RMS), coefficient of determination (R^2), and the number of data points (n) for the model $\ln I = a + b \ln L$ where I is volume (cubic millimetres) of seston ingested per larvae per minute and L is body length (millimetres).

Species and date	a (SE)	b (SE)	RMS	R^2	n
S. venustum/verecundum					
May 13, a.m.	−12.706	3.215	0.678	0.72	87
	(0.191)	(0.218)			
May 13, p.m.	−12.611	3.331	0.707	0.81	99
	(0.195)	(0.163)			
May 14, noon	−12.142	3.411	0.387	0.91	98
	(0.146)	(0.110)			
S. tuberosum					
May 13, a.m.	−12.323	3.688	0.363	0.80	81
	(0.243)	(0.204)			
May 13, p.m.	−11.867	3.027	0.380	0.68	78
	(0.291)	(0.240)			
May 14, noon	−11.672	3.331	0.270	0.85	80
	(0.185)	(0.157)			
S. decorum					
Combined	−11.750	3.003	0.351	0.83	46
	(0.317)	(0.205)			

TABLE 4. Discharge, seston concentration, and abundance of simuliids in gutter and stream experiments. Values in parentheses are the 95% confidence intervals for the mean.

Experiment	Discharge (L/min)	Input [seston] (μg/L)	Output [seston] (μg/L)	Biomass (g DM)	Number (thousands)
Gutter					
May 13, a.m.	2.665	886	699	2.56	33
		(780–992)	(589–809)	(2.27–2.77)	(29–37)
May 13, p.m.	2.665	658	423	2.20	23
		(603–713)	(309–537)	(2.02–2.42)	(21–26)
May 14, noon	2.975	1010	665	2.64	22
		(885–1135)	(584–746)	(2.37–2.86)	(19–24)
Stream					
May 13, a.m.	12 000	883	805	3131	34 735
		(762–1004)	(712–897)	(1512–7109)	(20 180–66 576)
May 13, p.m.	10 500	670	596	3131	34 735
		(615–725)	(482–710)	(1512–7109)	(20 180–66 576)
May 14, noon	9 300	1014	927	3131	34 735
		(894–1134)	(839–1015)	(1512–7109)	(20 180–66 576)
May 15, a.m.	8 070	1011	1129	0	0
		(879–1143)	(1056–1199)		
May 15, p.m.	7 680	1604	1663	0	0
		(1404–1804)	(1575–1751)		
May 16, noon	7 290	1085	1181	0	0
		(913–1257)	(1042–1320)		

section was detected. Larvae in the section ingested between 32 and 55% of the seston flux, corresponding to between 0.8 and 1.4% of the flux per metre of stream.

After black fly removal, there was an apparent production of seston in the studied section as indicated by the negative assimilation. However, confidence intervals ranged again from negative to positive, and the gain of seston was not significant at the 5% level.

Although the larvae ingested between 32 and 55% of the seston passing through the section, there was no significant change in seston flux between the top and bottom of the section for any experiment in the stream. This may reflect our sampling design, since we collected only 12 replicate samples whereas a posteriori calculations revealed that between 25 and 35 replicate samples would have been required on average to detect the predicted 6–14% difference in flux with 80% probability. However, when the results of replicated experiments are combined (Fisher 1954), the average 8.7% loss of seston between upstream and downstream when larvae were present is significant (G-test, $P = 0.035$), Conversely, after pooling of the measurements after the larvicide treatment, production of seston in the stream section after the larvicide treatment is almost, but not quite, significant (G-test, $P = 0.065$). The average production of seston in the stream section after the insecticide

TABLE 5. Results of the gutter experiments.

	May 13, a.m.	May 13, p.m.	May 14, noon
Water temperature (°C)	14	16	18
Mean individual mass (μg)	77	96	123
Specific ingestion rate (μg·mg DM^{-1}·min^{-1}) (95% CI)	1.13 (0.96–1.33)	1.13 (0.96–1.27)	1.53 (1.32–1.74)
Specific assimilation rate (μg·mg DM^{-1}·min^{-1}) (95% CI)	0.194 (0.06–0.33)	0.284 (0.18–0.43)	0.388 (0.24–0.53)
Assimilation efficiency (%) (95% CI)	17.2 (6–31)	25.1 (15–39)	25.3 (16–36)

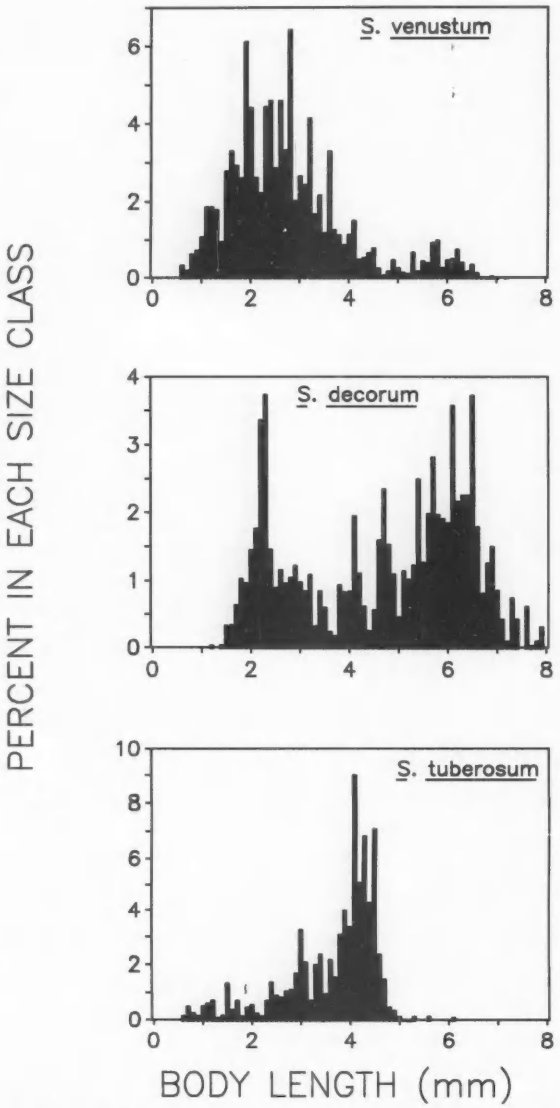

FIG. 5. Size distribution of the three dominant species of simuliids in the outlet of Petit Lac Fraser.

TABLE 6. Predicted and observed percent of seston flux ingested and assimilated by simuliids in the first 40 m of Petit Lac Fraser outlet (95% CI).

Date	Predicted % of flux ingested	Predicted % of flux assimilated	Observed % of flux assimilated
May 13, a.m.	32 (17–65)	6 (2–14)	9 (−5–20)
May 13, p.m.	51 (24–107)	13 (6–32)	9 (−5–23)
May 14, noon	55 (30–123)	14 (7–34)	8 (−6–19)
May 15, a.m.	0	0	−12 (−28–2)
May 15, p.m.	0	0	−4 (−18–8)
May 16, noon	0	0	−9 (−32–7)

treatment was 1 kg DM/d. Considering that about 3 kg DM of larvae were killed by the larvicide and that their bodies gradually decomposed and drifted out of the section during about 3 d, the measured seston production in the section may be attributable to the drifting fragments of dead larvae.

Impact of Filter-Feeders on Seston Transport

Our estimates of relative seston utilization per metre of stream by simuliid larvae are 1–3 orders of magnitude higher than values reported for populations of net-spinning caddisflies. Three factors may explain the difference. First, our estimates come from a stream where the potential impact of larvae on seston transport is higher than in most sites for which estimates are available, but still within the plausible range for small lake outlets (Fig. 6). Second, specific ingestion rates of filter-feeding caddisflies reported in these studies were about 10 times lower than those observed in this study and in other studies on simuliids (Fig. 7). Finally, most published estimates were made on an annual basis, whereas the ones presented here are for a short period when discharge was low and standing stock was high.

In the typical lake outlet systems of southern Quebec, black fly populations can ingest a significant portion of the seston flowing out of the lake during periods of high standing stock. In these cases, food is probably limiting growth of larvae, and coexisting species would benefit from a temporal segregation of their period of high energy demand. This may explain why the size distributions of the three dominant species are offset.

Periods of low discharge can be a threat to simuliids, not only because of a decrease in proper habitat, but also because

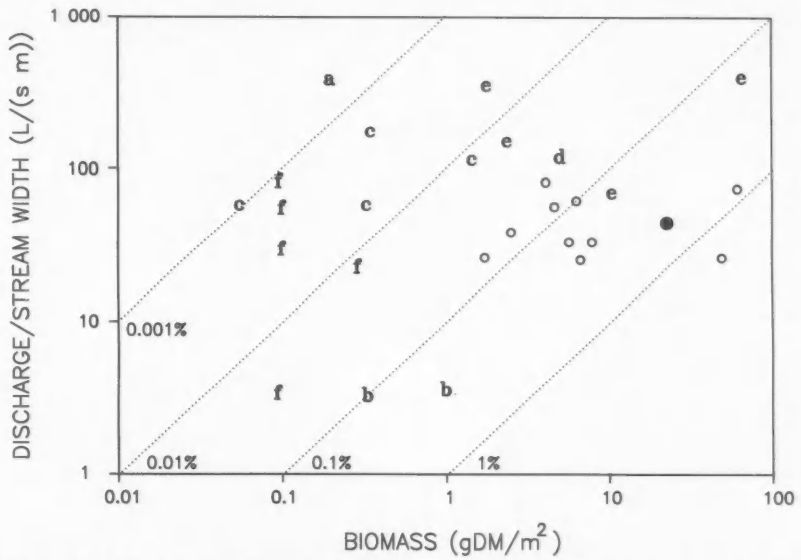

FIG. 6. Response surface of the percent seston flux ingested per linear metre of stream as a function of biomass of filter-feeders and discharge per metre of stream width. The lines are calculated assuming a specific ingestion rate of 1.5 μg·mg DM^{-1}·min^{-1} and a seston concentration of 2 mg DM/L. Superimposed are the conditions represented in studies from the literature (letters), data from lake outlets of southern Quebec (open circles, A. Morin unpubl. data), and the conditions at the outlet of Petit Lac Fraser (solid circle, this study). Note that most literature data come from sites where filter-feeders impact on seston is potentially less important than in southern Quebec lake outlets. Data from (a) Benke and Wallace (1980), (b) Haefner and Wallace (1981), (c) Ladle et al. (1972), (d) McCullough et al. (1979), (e) Parker and Voshell (1983), and (f) Ross and Wallace (1983).

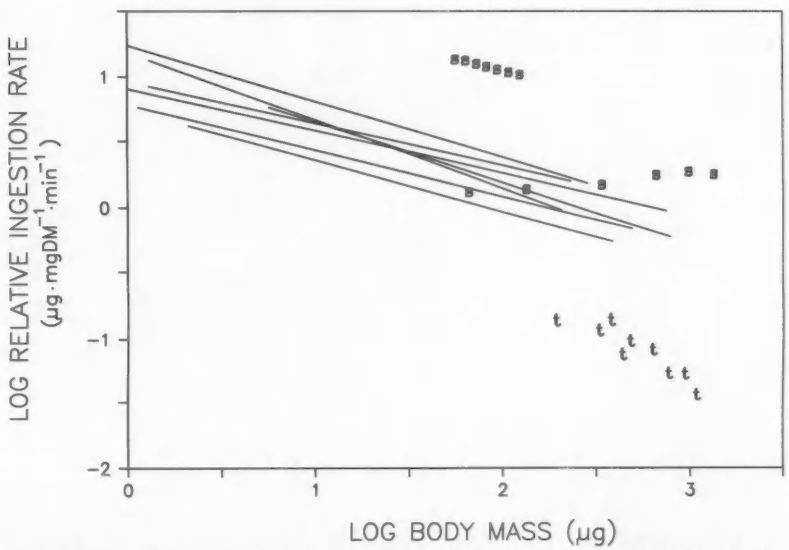

FIG. 7. Specific ingestion rates of stream filter-feeders in relation to their body mass. Lines are solutions to the equations obtained in this study by combining the body mass –, ingestion rate –, and density of gut contents – length regression equations. Literature data for simuliids (s) and for trichopteran (t) populations. Data from McCullough et al. (1979), Wotton (1978), Benke and Wallace (1980), Haefner and Wallace (1981), and Ross and Wallace (1983). Note that for similar body mass, specific ingestion rates of simuliids are about 10 times larger than those of trichopterans.

of the reduction of the food supply. Droughts in late spring and early summer, when simuliid populations attain their highest standing stock, will intensify food limitation in rich sites and probably increase mortality.

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Accuracy of Pup Classifications and Its Effect on Population Estimates in the Hooded Seal (*Cystophora cristata*)

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Stenson, G. B., and R. A. Myers. 1988. Accuracy of pup classifications and its effect on population estimates in the hooded seal (*Cystophora cristata*). Can. J. Fish. Aquat. Sci. 45: 715–719.

Information from the classification of age-specific developmental stages has been used to adjust aerial survey estimates of pup production in a number of species of seals, including the hooded seal (*Cystophora cristata*). We test the assumption that hooded seal pups were accurately and consistently classified according to developmental stage and examine the consequences of misclassifications upon adjusted population estimates. We determined overall misclassification rates, the effect of survey height on classifications, and interobserver variability. At ice level, misclassifications rates were low (<3%). From an altitude of 30 m, newborn pups could not be classified correctly and misclassification rates for the two other stages of attended pups varied between 6.4 and 21.3%. There was no evidence of an overall bias in classifications or differences among observers although there was a significant interaction between day and stage. Individual pups appear to have been misclassified independently by each observer. Under actual survey conditions, observers classified a similar proportion of pups into each recognizable stage. The misclassification rates we observed did not significantly alter the previous population estimate. Methods for improving the current survey design include modifying classification criteria, providing observers with a period of on-ice training, and reducing the width of survey transects.

L'information obtenue de la classification des stades de développement selon l'âge a été utilisée pour l'ajustement des estimations par levé aérien de la production de nouveau-nés chez un certain nombre d'espèces de phoques, y compris le phoque à capuchon (*Cystophora cristata*). Les auteurs vérifient l'hypothèse que les nouveau-nés du phoque à capuchon ont été précisément et uniformément classés selon le stade de développement et examinent les conséquences d'une classification erronée sur les estimations ajustées de la population. Ils ont ainsi déterminé les taux globaux de classification erronée, l'incidence de l'altitude des levés sur les classifications et la variabilité entre observateurs. Au niveau de la glace, les taux de classification erronée étaient faibles (<3 %). D'une altitude de 30 m, les nouveau-nés ne pouvaient être classés correctement; les taux de classification erronée des deux autres stades de nouveau-nés accompagnés de leur mère ont varié de 6,4 à 21,3 %. Il n'y avait aucun signe de biais global des classifications ou aucune différence entre les observateurs, bien qu'il y avait une interaction significative entre le jour et le stade. Chaque nouveau-né semble avoir été indépendamment mal classé par chaque observateur. Selon les conditions réelles de levés, les observateurs ont classé une proportion semblable de nouveau-nés selon chaque stade identifiable. Les taux de classification erronée observés n'ont pas nettement modifié l'estimation précédente de la population. Les méthodes d'amélioration de la conception des levés en cours comprennent la modification des critères de classification, une période de formation des observateurs sur la glace et la réduction de la largeur des transects de levés.

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Aerial survey techniques are commonly used to estimate pup production in a number of pinnipeds (e.g. Lavigne 1976; Radford et al. 1978; Hay et al. 1985; Bowen et al. 1987). However, determining total pup production is often difficult because in many species, individual pups may leave the whelping patch before all of the births are completed. Therefore, during any single survey, some pups may not be present because they have yet to be born or they have already left the whelping concentration. One method to correct a single survey estimate of pup production is to use a model which estimates the temporal distribution of births in the population. Such a model uses information on the duration of distinct age-specific developmental stages and the changes which occur in the proportion of pups in each stage over the entire period of births. Such models have been used successfully to correct aerial survey estimates of grey seals (*Halichoerus grypys*) (Radford et al. 1978), harp seals (*Phoca groenlandica*) (Myers and Bowen 1986), and hooded seals (*Cystophora cristata*) (Bowen et al. 1987) and are applicable to any species where only part of the population is present to be surveyed at any one time.

In the hooded seal, it is important to correct any estimate of pup production for the distribution of births. In the northwest Atlantic, births may occur on the pack ice from mid- to late March (Kovacs and Lavigne 1986). Individual pups, however, are present for only a short time and may leave the ice after weaning, as early as 4 d after birth (Bowen et al. 1985). Correcting for the temporal distribution of births, Bowen et al. (1987) increased their initial aerial survey estimate by more than

TABLE 1. Error rates of individual observers classifying hooded seal pups from an altitude of 30 m.

Date	Stage	*N*	Proportion of pups misclassified		
			Obs. A	Obs. B	Obs. C
March 16	2	12	0.083	0.167	0.083
	3	12	0.25	0.25	0.083
March 17	1	2	1.0	0.50	1.0
	2	40	0.05	0.20	0.05
	3	5	0.20	0.20	0.20
March 18	2	23	0.304	0.261	0.261
	3	14	0.071	0.071	0
Total	1	2	1.0	0.50	1.0
	2	75	0.133	0.213	0.120
	3	31	0.161	0.161	0.064

50%. Therefore, considering the importance of this correction factor to the estimate of total pup production, it is important that the assumptions of the model used to estimate the temporal distributions of births be tested. One assumption of the model used by Bowen et al. (1987) is that all pups were correctly classified according to developmental stage. This involved classifying pups from a moving helicopter at a height of 30 m (Bowen et al. 1987). The purpose of this paper is to test this assumption experimentally by determining misclassification rates, the effect of survey altitude, and the interobserver variability in stage classification. We also determine if the current model is robust to realistic levels of misclassification.

Methods

Two experiments were conducted in the Gulf of St. Lawrence in March 1986 to examine errors in classifying hooded seal pups. Pups were classified by three observers: two (B and C) with previous field experience with hooded seals and one (A) without. Each observer examined photographs and slides of pups in the laboratory to become familiar with the developmental stages defined by Bowen et al. (1987):

Stage 1: Newborn — skin in loose folds along flanks, fur saturated to wet, entire pelage yellowish, awkward body movements, mother present.

Stage 2: Thin blueback — ventrum white, neck well defined, trunk conical, mother present.

Stage 3: Fat blueback — ventrum white, neck not clearly distinguishable, trunk fusiform, mother present.

Stage 4: Solitary blueback — as in fat blueback but mother not present.

On March 16, a small concentration of whelping hooded seals was located at 46°40′N, 62°12′W, approximately 40 M (1 M = 1.852 km) south of the Magdalen Islands. Aerial observations were conducted from a Bell 206B helicopter in a manner similar to that used by Bowen et al. (1987). Flying altitude was 30 m and flying speed was approximately 65 km/h. Tape marks were placed on each side window to delimit a 100-m strip at an altitude of 30 m.

Before beginning the experiments, a series of pups was examined and the classification criteria used by Bowen et al. (1987) were found to be inappropriate for use from the air. The criteria used in this study were modified slightly; stages 1 and 4 remained the same, but stages 2 and 3 were differentiated solely by the presence or absence of a visible neck.

TABLE 2. Number of stage 2 and stage 3 hooded seal pups correctly classified by each observer from an altitude of 30 m.

Date	Stage	Correctly classified by								Total
		ABC	AB	AC	BC	A	B	C	None	
March 16	2	9	1	1				1		12
	3	8		1	1			1	1	12
March 17	2	27	2	8	3					40
	3	3		1			1			5
March 18	2	9	2	4	4	1	2		1	23
	3	12		1	1					14
Total	2	45	5	13	7	1	2	1	1	75
	3	23		3	2		1	1	1	31

Results

Error Rates and the Effect of Survey Height

To estimate the overall error rates and the effect of survey height, individual attended pups (stages 1–3) were classified from an altitude of 30 m and again near ice level. Pups were chosen by the pilot who presented the pup to each observer so that it was visible within the marked strip width. After each observer independently classified the pup, it was then observed from the helicopter while hovering just above ice level (1–3 m) and classified a second time. Only after these two classifications were completed was the developmental stage of the pup discussed between the observers and a consensus ("true stage") reached. To obtain a variety of pups in each developmental stage, this experiment was conducted over a 3-d period from March 16 to 18. Unattended pups were not included in this experiment because the absence of an adult female was an unambiguous criterion for stage 4 pups.

Each observer examined 108 pups. Although the percentage of pups in each of the three stages varied by day, in total, 1.8% were in stage 1, 69.4% in stage 2, and 28.7% in stage 3 (Table 1).

The observers agreed on the classifications of nearly all of the pups which were examined at ice level. The two stage 1 pups encountered were classified correctly and error rates for stage 2 and stage 3 pups were 2.6 and 1.1%, respectively. Most of the stage 2 and stage 3 pups misclassified were considered to be transitional between the two stages.

From an altitude of 30 m, stage 1 pups (newborns) were correctly classified only once (Table 1). Because this stage is difficult to correctly classify and was estimated by Bowen et al. (1987) to last for a very short time (approximately 3 h), we recommend that the newborn stage not be used when classifying pups from the air. It can, however, be used if classifications are made at ice level.

Misclassification rates for stage 2 and stage 3 pups were higher at 30 m than those made at ice level. Overall misclassification rates for stage 2 pups ranged between 12.0 and 21.3%, while misclassification rates for stage 3 pups ranged between 6.4 and 16.1% (Table 1). However, the most striking pattern that emerges from an examination of Table 1 is that the misclassification rates for stage 2 pups appear to be less than

the misclassification rates of stage 3 for March 16 and 17, but the reverse occurs for March 18. The numbers of stage 2 and 3 pups misclassified were modeled by a logit transformation of the data (McCullagh and Nelder 1983) and estimating a model with stage, observer, day, and interaction effects using maximum likelihood methods. There was no significant effect of stage ($P = 0.78$), indicating that there is no difference in misclassification rates between the two stages. Similarly, there was no significant effect of day ($P = 0.97$) or observer ($P = 0.10$). However, there was a significant interaction between day and stage ($P = 0.02$). Thus, although there was no bias between stage 2 and 3 misclassifications in the sense that there was no stage effect, there was a significant interaction between day and stage due to the reversal in misclassification rates on March 18.

The reason for the increase in stage 2 misclassifications (and decrease in stage 3 misclassifications) on March 18 is puzzling. The increase in stage 2 misclassifications on March 18 may be due to an increase in the proportion of pups which were transitional between the two stages and thus difficult to correctly classify. However, if transitional pups account for a large proportion of those misclassified, then we would expect that certain individual pups would tend to be misclassified more frequently. Thus, there should be a greater proportion of pups that were misclassified by two or more observers than would be expected if misclassifications were independent among observers. The hypothesis was checked by fitting a Poisson model to the data in Table 2 in which days and the two stages were combined (see Bishop et al. 1980, p. 316). The results are as follows:

	Number of observers misclassified			
	0	1	2	3
Observed frequency of seals	68	30	6	2
Poisson expected frequency	67.2	30.6	7.0	1.2

This results in a χ^2 of 0.62, indicating a good fit of the Poisson model. We cannot conclude from this analysis that the tendency of observers to misclassify the same pup is of any importance. (Note that the expected frequency for the last stage is actually for three or more observers.) The analysis was repeated separating the data by stage and day of observation with similar results. Therefore, there is no evidence that the misclassification rates were caused by individual transitional pups being misclassified.

Although stage 4 pups were considered to be readily classified by the absence of an attending female (Bowen et al. 1987), errors in identifying this stage could occur in areas with high densities of seals if a wandering solitary pup was mistaken for the attending stage 3 pup of a nearby adult. Because twinning has not been reported (Kovacs and Lavigne 1986), two pups associated with a single female would not result in an appreciable error; one pup would be considered solitary and the other classified as the attending pup. However, if the solitary pup was in close proximity to an unattended female (i.e. before birth or after weaning), it could result in an increased estimate of the number of stage 3 pups. A similar error could occur if the solitary pup was associated with a male that was mistaken for being female. Because hooded seals are sexually dimorphic, this potential error can be reduced by conducting the surveys at a speed slow enough to allow for the verification of the sex of the attending adults.

TABLE 3. Interobserver variability: numbers of pups classified and percentage of identifiable pups in each developmental stage during 10 transects.

	No. of pups			Stage (%)		
Observer	Total	Unidentified	Identified	2	3	4
A	116	11	105	82.8	16.2	1.0
B	128	32	96	78.1	19.8	2.1
C	127	24	103	78.6	18.4	2.9

Interobserver Variability

A second experiment was conducted on March 17 to determine the variability in pup classifications among observers under actual survey conditions. Using a Loran C navigational system, 10 east–west transects were flown across the entire whelping patch at an altitude of 30 m. All pups observed within the marked strip (100 m) were classified into one of the four stages. Pups that could not be classified were counted as unknown. Each transect was then flown in the opposite direction so that all observers examined the same study area. Observers A and B examined the transect on the same pass and observer C viewed it on the other.

The total number of pups in each developmental stage observed by each observer on the 10 survey transects was similar (Table 3). The greatest variability was in the number of pups which could not be classified (unknown). The classifications of two experienced observers (B and C) were similar, while the inexperienced observer (A) tended to classify more pups as stage 2 and fewer as unknowns.

Only the proportion of pups in a recognizable stage are considered when corrections are made to survey results (Bowen et al. 1987). If the unknown pups are ignored, the three observers classified a similar proportion of pups into each recognizable stage (Table 3).

The consistency of the classifications may be increased by improving the method used to delimit the viewing area and by narrowing the strip. The use of a single set of tape marks to delimit the viewing area resulted in significant changes in the effective strip width when the observer changed his sitting position slightly. This difference in viewing area may account for some of the difference between observers A and B who were examining the field on the same pass. A narrower strip would eliminate the pups which were far away from the observer and difficult to classify owing to the low angle of view. It would also decrease the maximum distance between the animal and the observer, thereby increasing the sightability (Caughley 1974; Caughley et al. 1976; Estes and Gilbert 1978). Finally, a narrower strip would give more time to view individual pups, which increases the accuracy of aerial surveys (Caughley 1974; Wartzok and Ray 1975). Having more time to classify pups may be particularly important in areas such as northeastern Newfoundland (the "Front"), where pup densities are higher than those in the Gulf.

Although reducing the width of the viewing strip would result in fewer pups being classified per transect, it is unlikely to significantly reduce the accuracy of the estimate of stage composition of the whelping patch. This is because hooded seal pups of similar stage tend to be associated (Bowen et al. 1987), and thus the major source of variability in the estimate is due to differences among geographical regions within the whelping patch. Therefore, the accuracy of the stage composition estimate is only slightly improved if a large number of pups are

classified on individual transects, providing the entire patch is properly sampled to determine the extent of geographical differences.

Discussion

Accurate classifications of developmental stages are possible at ice level. By descending upon a random series of pups without actually landing, accurate classifications of a number of pups can be made reasonably quickly. On-ice classifications have been found to be the most effective method of obtaining stage composition information for harp seals (Myers and Bowen 1986). However, classifications from a greater altitude provide some advantages over those at ice level: a larger sample size can be obtained; systematic transects can be flown and data collected for an estimate of population abundance; and the mother–pup pairs are not disturbed. Therefore, aerial transects are preferable if misclassification rates are acceptable.

Under the favorable conditions of experiment 1, stage 2 and 3 pups can be identified from an altitude of 30 m with reasonable error rates. However, the results of these experiments suggest that stage 1 should not be used as a category. Also, it is important that classification criteria be easily recognized. The criteria Bowen et al. (1987) used to distinguish stage 2 and stage 3 pups were reduced to the presence or absence of a visible neck. This criterion was chosen in an attempt to make the classification more consistent among observers and was found to work reasonably well. We suggest that this criterion be used in future surveys.

The initial pup classifications made before the experiments began were not consistent among the observers, and consequently, a period of training was needed to standardize the observers before the experiments began. It was necessary to view at least two dozen pups of various developmental stages from the air and at ice level before reasonably consistent classifications could be made. After training, all three observers had similar error rates (experiment 1), indicating that even inexperienced observers can learn quickly and effectively with the proper period of training. Before the training period, the variability in classifications was similar for observers with or without previous experience with hooded seals; experienced observers without current practice were no more accurate than inexperienced observers. A similar problem was recognized by LeResche and Rausch (1974) who found that experienced observers with current experience identified significantly more moose during an aerial survey than experienced observers without current experience. Therefore, before future surveys are undertaken, both experienced and inexperienced observers should have a training period during which pups are examined under field conditions.

Effect of Misclassifications on Estimates of Population Size

There are two effects of pup misclassification that are of concern: bias and variance. There was little bias in the estimates of proportions of pups in stages 2 and 3; overall, this bias was not statistically significant and varied among the observers. Nevertheless, this bias should be considered in future estimates of pup production. The second effect of misclassification is to increase the variance in the estimates of the proportion of pups in each stage. If errors occur in classifying stage 2 and 3 pups only, an observer who mistakes stage 2's and stage 3's at an equal rate of 20% will estimate that a population consisting only of stage 2 pups will contain 20% of stage 3 pups. Thus, if the two stages are not present in equal proportions, this misclassification will result in a biased estimate of the proportions of pups present. The effects of variance are shown in Table 4. Observer B recorded a significantly larger ($P < 0.001$) number of stage 2 pups than were actually present on March 17. Although the error rates for stage 2 and 3 pups were identical for observer B on March 17 (Table 1), the disproportionately large number of stage 2 pups present resulted in this effect. This effect can be corrected for if the misclassification rates of each observer are known. Thus, it is important to perform experiments similar to this prior to future surveys to determine error rates for each observer. However, if misclassification rates for each observer are determined prior to the beginning of the experiment, they may not be valid for all days, since error rates were observed to vary from day to day (Table 1).

One method to modify a model which corrects pup production based on the temporal distribution of births to account for errors in classification is to incorporate errors in stage determination in the manner that Fournier and Archibald (1982) suggested for age misclassification. However, this method assumes that misclassifications occur independently of how long a pup has been in a stage (i.e. are independent of the age of the pup). If pups that are near a stage transition are classified incorrectly more often than other pups, this technique will not be applicable.

A more useful method is to modify estimates of the probabilities of pup stage transition as a function of age (these transition probabilities allow the information on stage composition to be used to estimate the distribution of births over time). However, to do this, we would need observations from the air of known-age pups.

We tested the sensitivity of the conclusions in Bowen et al. (1987) by modifying the relative durations of stages 2 and 3. In the model described in Bowen et al. (1987) the stages were of stochastic duration where the sojourn time in each stage was described by a gamma distribution. For the sensitivity analysis, we reduced the mean duration of stage 2 by 10% and increased the mean duration of stage 3 by 10%. We held the shape param-

TABLE 4. Numbers of stage 2 and stage 3 pups classified by each observer from an altitude of 30 m. *Significantly different from true ($P < 0.05$); ***significantly different from true ($P < 0.001$).

	March 16		March 17		March 18		Total	
Observer	Stage 2	Stage 3	Stage 2	Stage 3	Stage 2	Stage 3	Stage 2	Stage 3
A	14	10	39	6	17	20	70	36
B	13	11	33	12***	18	19	64	42*
C	12	12	39	6	17	20	68	38
True	12	12	40	5	23	14	75	31

eter of the gamma distributions constant. The result was only a 1% increase in the estimate of the total number of pups born off southeastern Labrador in 1984. Similar results were obtained if the mean duration of stage 2 was increased and the mean duration of stage 3 decreased. We also tested the sensitivity of the results to misclassifying stage 1 pups. The data described in Bowen et al. (1987) were reanalyzed using a model that eliminated the newborn stage (stage 1); the observed stage 1 pups were reclassified as stage 2. There was less than a 1% change in the estimates of pup production in this case because of the small numbers of stage 1 pups observed in 1984.

If the error rates we identified are similar to those which occurred during the survey of Bowen et al. (1987), these changes have only a small effect on their estimates of hooded seal pup production. This is because the time pups spend in each stage was assumed to be stochastic. Furthermore, the stochastic duration pups spend in each stage was estimated from data in which the pup age was estimated with some error. This effect will mitigate the greater rate of misclassifying pups from helicopter surveys.

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Photosynthetic Carbon Metabolism by Phytoplankton in a Nitrogen-Limited Reservoir[1]

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Phytoplankton in the downlake epilimnion of Normandy Lake, a central Tennessee reservoir, responded to summer N deficiency by increasing relative rates of lipid synthesis from 10–15% up to 20–35% of the total photosynthetic C fixation. Phytoplankton in more N-sufficient areas of the reservoir (downlake in a metalimnetic chlorophyll peak and uplake near the river inflow) maintained lower rates of lipid synthesis, generally ≤10% of the total fixed C, throughout the summer. NH_4 enrichment of N-deficient phytoplankton inhibited photosynthesis and significantly depressed the high lipid synthesis rates; however, NH_4 enrichment had no effect on the phytosynthesis or lipid synthesis of N-sufficient phytoplankton. Our results document, for the first time, the occurrence of high lipid synthesis rates associated with the N limitation of natural phytoplankton assemblages. This relationship has previously been observed only in laboratory algal culture studies.

Le phytoplancton de l'épilimnion de la partie aval du lac Normandy, un réservoir du centre du Tennessee, réagissait à la carence estivale en N en augmentant le taux relatif de synthèse des lipides qui passait de 10–15 % à 20–35 % de la fixation photosynthétique totale du C. Le phytoplancton des zones plus riches en N du réservoir (en aval dans une zone métalimnétique riche en chlorophylle et en amont près du point de déversement de la rivière) maintenait des taux de synthèse de lipides inférieurs, généralement de ≤10 % du C fixé total, tout au long de l'été. L'enrichissement par NH_4 du phytoplancton carencé en N inhibait la photosynthèse et réduisait de façon significative le taux élevé de synthèse des lipides. Cet enrichissement n'avait cependant aucun effet sur la photosynthèse ou la synthèse des lipides du phytoplancton non carencé en N. Les résultats obtenus montrent, pour la première fois, l'existence de taux élevés de synthèse des lipides correspondant à la limitation en N d'assemblages phytoplanctoniques naturels. Cette relation n'avait antérieurement été observée qu'en laboratoire, au cours d'études portant sur des cultures d'algues.

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A quantitative understanding of the interactions between phytoplankton and nutrients is required for predicting the productivity of pelagic ecosystems. Nutrient availability (Fuhs et al. 1972), intracellular nutrient content (Droop 1974; Goldman 1980), and cellular ratios of critical nutrients (Donaghay et al. 1978; Rhee and Gotham 1980; Goldman 1980) have all been related to algal growth rate and physiological status in laboratory culture studies. Biochemical composition has also been correlated with algal nutrient status. A high protein content is often characteristic of high growth rates, while under nutrient limitation and reduced growth, carbohydrates generally become more abundant (Healey 1975). Under N starvation, many algae accumulate large amounts of lipids in culture (Fogg 1959; Shifrin and Chisholm 1981). Although both the nutrient content (Haug et al. 1973; Healey and Hendzel 1980) and the biochemical composition (Antia et al. 1963; Haug et al. 1973; Barlow 1980, 1982a) of suspended particulate matter have been used to describe the dynamics of phytoplankton communities, these measurements often prove to be relatively insensitive because of the methodological difficulty of distinguishing phytoplankton biomass from that of other microbes and from detritus (Sakshaug 1980).

Measurement of short-term physiological processes associated with photosynthetic metabolism is another way to examine phytoplankton–nutrient interactions. Recent studies have shown that phytoplankton photosynthesis can be depressed by N and P enrichment in short-term incubations when those nutrients are limiting (Falkowski and Stone 1975; Thomas et al. 1976; Healey 1979; Lean and Pick 1981; Lean et al. 1982; Turpin 1983). Lean and Pick (1981) concluded that a short-term depression of C fixation occurs under these conditions as a result of competition for ATP between nutrient uptake and C fixation and suggested that the observed depression in C fixation provides a potential tool for detecting nutrient limitation. Other investigators have employed ^{14}C uptake to measure the allocation of

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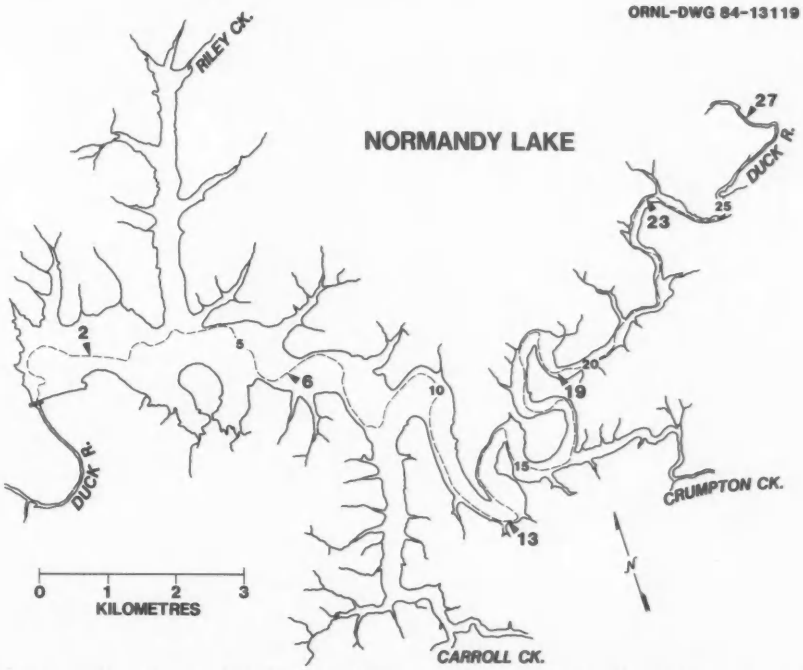

FIG. 1. Location of Normandy Lake and Duck River sampling stations. Sampling stations are marked with pointers and are numbered according to their distance (kilometres) from the dam.

recently fixed photosynthate to intracellular macromolecular pools (Morris et al. 1974; Konopka and Schnur 1980; Barlow 1982b). This methodology is particularly promising because the synthesis of certain photosynthetic end products may be closely related to phytoplankton growth rates and physiological status. We combined measurements of nutrient availability, photosynthetic responses to nutrient enrichment, and photosynthetic C metabolism patterns to investigate the metabolic responses of a reservoir phytoplankton community to the development of nutrient-deficient conditions along temporal and spatial gradients.

Study Site

Normandy Lake (Fig. 1), a reservoir located on a fourth-order reach of the Duck River in the Tennessee Central Basin, was impounded in 1976. The reservoir surface area and maximum volume are 1308 ha and 135×10^6 m^3, respectively, with the mean and maximum depths being 10 and 23 m. The dam has a hypolimnetic discharge at 20 m, and the mean annual retention time of the impoundment is 170 d. The reservoir water surface elevation ranges from a winter low of 263.7 m to a high of 266.7 m above mean sea level during the early summer. The Normandy Lake drainage basin is 505 km^2 in area, with the Duck River watershed above the reservoir accounting for 55% (277 km^2) of the total. Riley Creek (28 km^2), Carroll Creek (54 km^2), and Crumpton Creek (80 km^2) watersheds account for most of the remaining drainage area (Brown 1984). The Tennessee Central Basin is known for its rich deposits of phosphatic rock, but within the Duck River drainage, the phosphate deposits are mostly located downstream of Normandy Lake (Smith and Whitlatch 1940).

Our sampling stations were located along the longitudinal axis of the reservoir (Fig. 1). Station numbers correspond to their distance (kilometres) upstream from the dam (0 km) along the river channel. We sampled the entire water column seasonally at station 2. Mixed-layer samples were collected from a depth of 1–2 m at stations 2, 6, 13, 19, and 23 to determine longitudinal patterns within the reservoir. Samples from the inflowing river were obtained at station 27.

Methods

Normandy Lake was sampled nine times from February through October 1983; each sampling period lasted 2–4 d. Water temperature was measured with a Hydrolab 4011 unit. A Li-Cor quantum sensor was used to measure surface and subsurface photosynthetically active radiation (PAR) (400–700 nm). Water samples for nutrients were vacuum filtered (Whatman GFC), frozen, and later analyzed using a Technicon autoanalyzer (Stainton et al. 1977). Particulate P concentrations were determined as described by Stainton et al. (1977). Particulate C and N were measured with a Perkin Elmer 240b CHN analyzer. Chlorophyll *a* (Chl *a*) was determined spectrophotometrically on methanol extracts and corrected for pheophytin (Marker et al. 1980).

Enhancement of dark C uptake by NH_4 enrichment was used to detect phytoplankton N deficiency (Yentsch et al. 1977). Three or four replicate 130-mL glass bottles containing sample were enriched with NH_4Cl (final concentration 100 μg N·L^{-1} over ambient concentration) and incubated in the dark for 4 h. Unenriched samples served as controls. ^{32}P-PO_4 turnover times were measured over a 2-h time course (Lean 1973).

Photosynthetic C metabolism, defined here as the flow of photosynthate into intracellular macromolecular pools (polysaccharide, protein, lipid) and into a pool of soluble metabolites, was measured according to Li et al. (1980). Water was poured into transparent Pyrex bottles, ^{14}C-HCO_3 was added to a final concentration of 0.066–0.20 $\mu Ci \cdot mL^{-1}$ (1 μCi = 37 kBq) (depending on the phytoplankton biomass present in the reservoir when sampled), and the samples were incubated within the water column. After a 3- to 4-h incubation period, samples were filtered onto Gelman 934 AH filters, frozen at −20°C, and extracted within 2 wk. The extracted components were defined as soluble metabolites (methanol–water-soluble fraction), lipid (chloroform-soluble fraction), polysaccharide (hot 5% trichloroacetic acid-soluble fraction), and protein (remaining residue). Nucleic acids are also extracted with the polysaccharide fraction. The fractionation procedure recovered 99% (coefficient of variation = 5%) of the total particulate ^{14}C retained on the filter; coefficients of variation for the macromolecular fractions, expressed as percentages of the total C fixed, were 5.5% for protein, 5.6% for polysaccharide, 7.4% for lipid, and 8.1% for the soluble metabolites. Accumulation of ^{14}C in the macromolecular pools was linear for at least 6 h under constant, nonphotoinhibiting light intensities.

Nutrient enrichment experiments were conducted in a similar manner to the incubations described above except that the samples were enriched with 1-mL aliquots of K_2HPO_4 or NH_4Cl to a final concentration of 10 $\mu g\ P \cdot L^{-1}$ or 100 $\mu g\ N \cdot L^{-1}$. In July and September, nutrient enrichment effects on photosynthetic C metabolism were measured through time on 450-mL samples in 500-mL flasks using the same nutrient concentrations. Unenriched controls were run in parallel for all enrichment experiments, and the incubations were conducted in indirect sunlight.

Results

Seasonal Patterns of Nutrient Availability and Phytoplankton Carbon Metabolism in the Downlake Epilimnion

Seasonal changes in dissolved and particulate nutrient concentrations, algal biomass, the results of algal nutrient-deficiency assays, and patterns of photosynthetic C metabolism reflected the development of N-deficient conditions in the downlake epilimnion of Normandy Lake (at station 2) during the growing season (Fig. 2). NO_3-N concentrations decreased from high winter and spring values to undetectable levels in July. From July through October, total nitrogen to total phosphorus (TN:TP) and dissolved inorganic nitrogen to soluble reactive phosphorus (DIN:SRP) atomic ratios were <20 and <10, respectively, values indicative of N limitation (Sakamoto 1966; Forsberg et al. 1978). A virtual disappearance of NO_3-N early in the growing season has been observed in other N-limited lakes (Horne and Goldman 1972; Axler et al. 1982; Vincent et al. 1984). The mean concentration (and range) for SRP was 7 $mg \cdot m^{-3}$ (1–12 $mg \cdot m^{-3}$) and for NH_4-N was 9 $mg \cdot m^{-3}$ (1–14 $mg \cdot m^{-3}$) from July through September. Mean seston atomic ratios were 14 (range = 12–16) for C:N, 21 (19–23) for N:P, and 284 (256–339) for C:P during this same period. Between September 15 and October 12, the depth of the epilimnion increased from 6 to 11 m and SRP (15 $mg \cdot m^{-3}$) and NH_4-N (45 $mg \cdot m^{-3}$) became more abundant. Chlorophyll *a* concentrations also declined in the epilimnion during the summer, and particulate C : Chl *a* (weight:weight) were highest in July and August (Fig. 2).

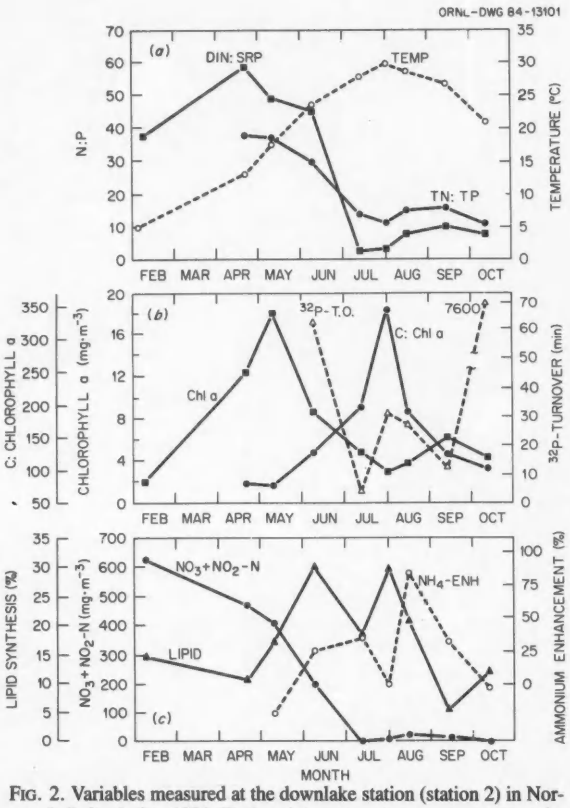

FIG. 2. Variables measured at the downlake station (station 2) in Normandy Lake during 1983. Each point represents measurements at the depth of light-saturated photosynthesis within the epilimnion (always within the upper 2 m). Chl *a* concentrations and lipid synthesis values represent the mean of measurements made on two or three consecutive days. (a) Temperature, total nitrogen to total phosphorus (TN:TP) atomic ratio, and dissolved inorganic nitrogen to soluble reactive phosphorus (DIN:SRP) atomic ratio; (b) Chl *a* concentration, carbon to Chl *a* ratio (by weight), and $^{32}PO_4$ turnover time (^{32}P-T.O.); (c) $NO_3 + NO_2$-N concentration, lipid synthesis (as a percent of the total photosynthetic carbon fixation), and NH_4 enhancement of dark C uptake relative to controls (NH_4-ENH).

NH_4 enrichment of samples significantly enhanced dark C uptake between June and September (with the exception of early August), but did not stimulate dark C uptake in May or October (Fig. 2). These results provide additional evidence for summer N limitation of the downlake epilimnetic phytoplankton in Normandy Lake (Vincent 1981). Phosphorus turnover times decreased (to <30 min) during July–September relative to those measured in June and October (>1 h), suggesting that components of the phytoplankton community were also P limited. However, Chl *a*-specific alkaline phosphatase activity (measured in July and September) was low in Normandy Lake relative to values reported for P-limited, upper Tennessee River basin reservoirs (Elser and Kimmel 1985).

The flow of algal photosynthate into lipid synthesis exceeded 20% of the total photosynthetic C fixation from May through August and >30% of the total in June and early August (Fig. 2c). During the May–August period, absolute and relative rates of lipid synthesis ($\bar{x} \pm SE$ = 0.97 ± 0.12 mg C·mg Chl $a^{-1} \cdot h^{-1}$ and 25.4 ± 0.4% of the total ^{14}C fixed,

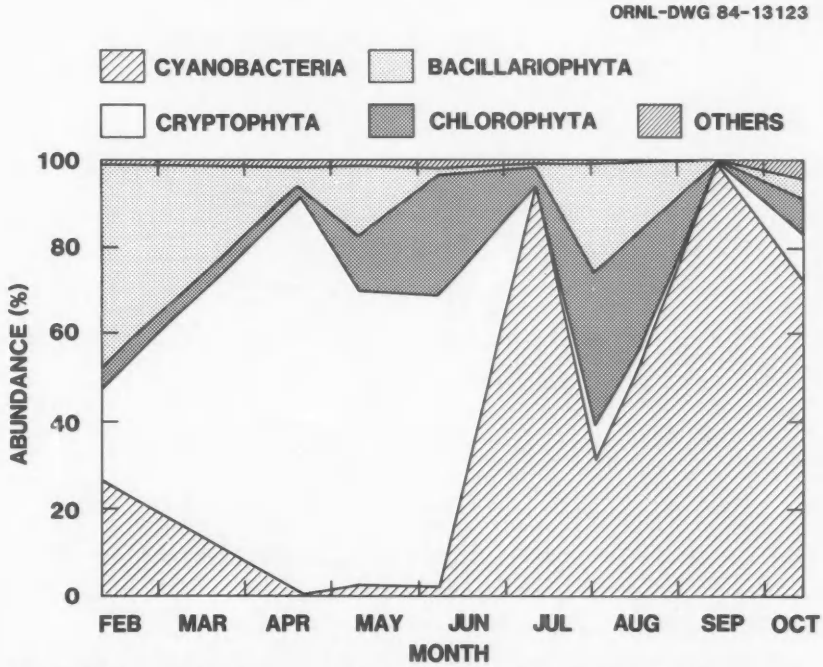

FIG. 3. Relative abundance (as a percent of total cell numbers) of the major phytoplankton groups in the epilimnion (average of samples from the upper 4 m at station 2) of Normandy Lake in 1983.

respectively; $n = 11$) for downlake epilimnetic phytoplankton during light-saturated photosynthesis (P_{max}) were significantly greater ($p < 0.001$) than corresponding values ($\bar{x} \pm SE = 0.52 \pm 0.04$ mg C·mg Chl $a^{-1}\cdot h^{-1}$ and $16.3 \pm 0.01\%$ of the total ^{14}C fixed; $n = 98$) from a larger data set of phytoplankton C metabolism at P_{max} from a broad range of chemical, physical, and trophic conditions (A. W. Groeger and B. L. Kimmel unpubl. data). The period of high lipid synthesis corresponded to decreasing or low N availability, as indicated by seston chemistry, nutrient concentrations, and NH_4 enhancement of dark C uptake (Fig. 2).

The taxonomic composition of the phytoplankton community at station 2 also exhibited distinct seasonal transitions (Fig. 3). Cyanobacteria (mostly *Microcystis sp.*) increased from <5 to >90% of the cells present between the June and July sampling dates, corresponding to the disappearance of NO_3-N. Cyanobacteria often dominate the phytoplankton of lakes with low TN:TP ratios (Smith 1983). However, cyanobacterial genera known as N_2 fixers under aerobic conditions never accounted for >1% of the cells present in Normandy Lake, suggesting that N_2 fixation was probably not an important pathway for phytoplankton N assimilation.

Longitudinal and Vertical Patterns of Nutrient Availability and Photosynthetic Carbon Metabolism

Marked longitudinal gradients in near-surface seston chemical composition, dissolved nutrient concentrations, and phytoplankton photosynthetic C metabolism occurred in Normandy Lake during July and August (Fig. 4). Sestonic C:N and C:Chl *a* ratios generally increased within the reservoir from uplake to downlake (from station 23 to station 2) and were also high in the inflowing river at station 27. Both near-surface Chl *a* concentrations and phytoplankton productivity were high uplake and decreased downlake. SRP and $NO_3 + NO_2$-N concentrations were high in the river, but declined rapidly upon entering the reservoir where $NO_3 + NO_2$-N became undetectable. The extent of NH_4 enhancement of dark C uptake also increased downlake, showing, along with increasing C:N and the disappearance of NO_3-N, that algal demand for inorganic N increased with distance downstream from the nutrient-rich river inflow (Fig. 4). During this period, all reservoir stations were within ± 1° water temperature of each other (28–29°C), with the inflowing river (station 27) being slightly cooler (26°C).

Lipid synthesis accounted for about 10% of the photosynthetically fixed C at station 23 in July and August. This fraction increased progressively downlake to 24–26% of the total photosynthate at station 2, corresponding to decreasing N availability (Fig. 4). The ratio of polysaccharide to protein synthesis was greater uplake (except at station 23 in July) than downlake, although the difference was less distinct in August. Carbon flow to the soluble metabolite pool consistently made up about 10% of the total C fixation at all stations (Fig. 4).

Vertical profiles of $NO_3 + NO_2$-N, phytoplankton biomass and productivity, and photosynthetic C metabolism at station 2 on August 2 and 17 also indicate a relationship between N deficiency and high rates of lipid synthesis by Normandy Lake phytoplankton (Fig. 5). $NO_3 + NO_2$-N concentrations were low or undetectable in the epilimnion and relatively high in the metalimnion or hypolimnion. Epilimnetic Chl *a* concentrations were also low (<5 mg Chl $a\cdot m^{-3}$), but a distinct Chl *a* peak occurred in the metalimnion at 5 m. Volumetric primary productivity profiles showed apparent near-surface photoinhibition, an epilimnetic peak, and a metalimnetic maximum corresponding to

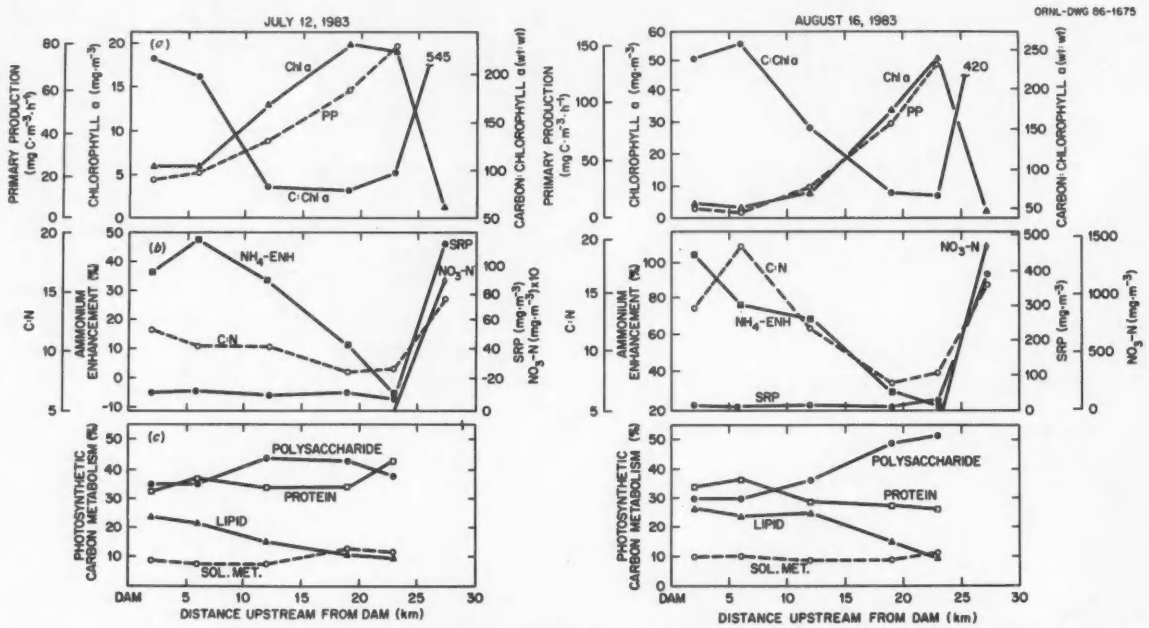

FIG. 4. Variables measured along the longitudinal axis of Normandy Lake on July 12 and August 16, 1983. (a) Chl *a* concentrations, carbon to Chl *a* ratios (by weight), and phytoplankton primary production (PP); (b) soluble reactive phosphorus (SRP) concentration, $NO_3 + NO_2$-N concentration, particulate carbon to nitrogen (C:N) atomic ratio, and NH_4 enhancement of dark C uptake (NH_4-ENH); (c) photosynthetic C metabolism.

the Chl *a* peak at 5 m. The euphotic zone depth (the depth to which 1% of surface light penetrated) was 7 m on both sampling dates.

Patterns of photosynthetic C metabolism also changed significantly with depth (Fig. 5). The absolute and relative rates of C flow into the polysaccharide pool were much higher in the metalimnetic (5 m) phytoplankton assemblage than in the epilimnetic phytoplankton. In the 5-m sample, absolute rates of protein and soluble metabolite synthesis also increased at least 100% over the 4-m sample, but on both dates, lipid production actually decreased between these two depths. Lipid synthesis accounted for 22–35% of the photosynthetically fixed C in the epilimnion, but was <10% of the total C fixation in the metalimnetic Chl *a* peak.

Nutrient Enrichment Effects on Photosynthetic Carbon Metabolism

Nutrient additions to near-surface samples at station 2 in February and May had little or no effect on phytoplankton photosynthesis or photosynthetic C metabolism (Table 1). However, enrichment of July samples from stations located along the longitudinal axis of the reservoir resulted in the depression of photosynthetic C fixation by both NH_4 and PO_4 additions (Fig. 6; Table 1; data for the control treatment are also shown in Fig. 4). The greatest decreases in C fixation relative to the controls were produced by NH_4 additions to downlake samples, in which over half of the decrease resulted from reduced lipid synthesis. Inhibition of the relative rates of lipid synthesis was less at uplake stations (Table 1), and P enrichment had no significant effect on relative rates of lipid synthesis at any station (data not shown). Carbon flow into polysaccharide synthesis was usually reduced by both N (Fig. 6) and P (Table 1) enrichments. Absolute rates of protein and soluble metabolite synthesis were least sensitive to nutrient enrichments (Fig. 6). However, in the NH_4-enriched treatments, protein synthetis increased in relative importance at all five stations, with three of the increases being statistically significant (Table 1).

Epilimnetic samples from 0 and 3 m were collected at station 2 on July 13, and photosynthesis rates and photosynthetic C metabolism were determined for control, PO_4, and NH_4 enrichments over a 3-h time course. In the 3-m sample (the 0-m sample behaved in a similar fashion, Table 1), primary productivity was inhibited 24% by NH_4 enrichment after 3 h, with 69% of this inhibition resulting from reduced lipid synthesis (Fig. 7). Lipid synthesis accounted for about 25% of the fixed C in the control sample, but only 11% of the C fixation in the NH_4 treatment (Fig. 7). The NH_4 addition rapidly depressed lipid synthesis, being fully effective within 15 min. Polysaccharide synthesis was also significantly altered, causing the relative importance of protein synthesis to increase with NH_4 addition. In both 0- and 3-m samples, PO_4 enrichment reduced photosynthesis, with decreased polysaccharide synthesis accounting for most of that reduction (Table 1). PO_4 enrichment did not reduce absolute rates of lipid synthesis in either sample (data not included in Table 1).

Nutrient enrichments were performed on samples from the epilimnion (1 m) and the metalimnetic Chl *a* peak (5 m) on August 3 to further examine the effect of nutrient enrichment on the photosynthetic C metabolism of nutrient-deficient phytoplankton (Fig. 8). Photosynthesis was significantly inhibited by NH_4 enrichment, but was unaffected by P enrichment at 1 m, where lipid synthesis accounted for 34% of the total C assimilated. Over 50% of the NH_4-induced depression in photosynthesis was due to reduced lipid synthesis. In the 5-m sample, where lipid synthesis accounted for only 7% of the total assimilated C, NH_4 addition had no effect on photosynthesis,

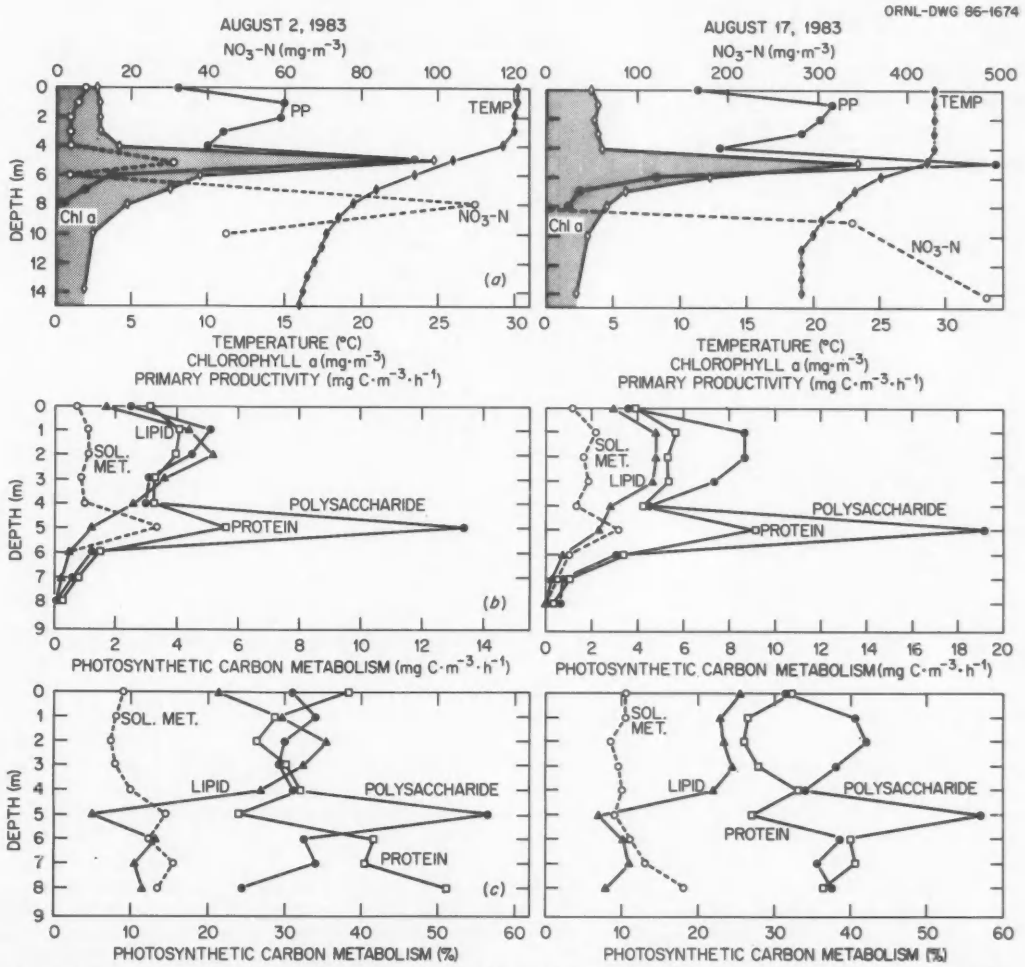

FIG. 5. Vertical profiles of variables measured at station 2 in Normandy Lake on August 2 and 17, 1983. (a) Chl *a* concentration, phytoplankton primary productivity (PP), $NO_3 + NO_2$-N concentration, and water temperature; (b) absolute rates of photosynthetic C metabolism; (c) relative rates of photosynthetic C metabolism. The 1% light level was at 6.5 and 7 m, respectively. Soluble metabolites = SOL. MET.

although P enrichment significantly reduced photosynthesis and decreased polysaccharide synthesis (Fig. 8). When rates of lipid synthesis were low in September, NH_4 enrichment had no effect on photosynthesis (Table 1).

Discussion

Because of the advective influence of the impounded river, reservoirs are often spatially heterogeneous environments that possess both horizontal and vertical gradients in environmental factors that control phytoplankton productivity (Gloss et al. 1980; Kennedy et al. 1982; Kimmel and Groeger 1984; Marzolf 1984; Kimmel et al. 1988). In Normandy Lake, longitudinal gradients in rates of phytoplankton lipid synthesis, in NH_4-enhanced C uptake in the dark, and in effects of nutrient enrichment on photosynthetic C metabolism provided evidence of algal physiological responses to the uplake-to-downlake decline in N availability.

Addition of a limiting nutrient to nutrient-deficient phytoplankton results in the depression of photosynthesis in short-term incubations (Falkowski and Stone 1975; Thomas et al. 1976; Healey 1979; Lean and Pick 1981; Lean et al. 1982; Turpin 1983). Therefore, the results of the nutrient-enrichment experiments provide evidence for summer N limitation of phytoplankton production in the downlake epilimnion of Normandy Lake. NH_4 enrichment of epilimnetic samples from station 2 resulted in a significant depression of photosynthetic C fixation in July and August. During the same period, rates of lipid synthesis by downlake epilimnetic phytoplankton were high (20–35% of the total C fixation), and these elevated rates of lipid synthesis were reduced by NH_4 enrichment. NH_4 additions did not depress photosynthesis rates in February, May, or September samples, in samples from the metalimnetic Chl *a* peak, or in samples from uplake stations in July. Three of four downlake epilimnetic P enrichments also depressed photosynthesis in July, a result that supports the P turnover time data in

TABLE 1. Phytoplankton productivity and photosynthetic carbon metabolism in control (unenriched, C), NH_4-enriched (N), and PO_4-enriched (P) samples from Normandy Lake, 1983.

Date	Station	Depth (m)	Dominant algal genera[a]	Phytoplankton productivity (mg C·m^{-3}·h^{-1})			Relative carbon flow (%)						
							Lipid		Protein			Polysaccharide	
				C	N	P	C	N	C	N	P	C	P
Feb. 9	2	0.5	A, L, O	4.3	4.5	4.4	18.0	17.2[c]	15.8	15.3	16.4	33.7	35.1
May 12	2	0.5	M, O, P	36.6	38.5	37.8	21.8	21.9	23.9	23.4	24.1	27.2	28.0
July 12	2	0.5	A, J	18.5	12.4[d]	17.4	23.7	9.0[d]	32.5	42.3[c]	36.4	34.6	29.1[c]
	6	0.5	A, J	21.4	17.2[c]	18.6[d]	21.8	13.5[c]	36.1	38.5	35.6	34.8	32.3
	13	0.5	A, E, H, J	33.8	28.7	30.1	14.6	10.4[c]	34.1	38.9[d]	32.3	44.1	42.9
	19	0.5	A, B, H	58.6	50.8	52.6	10.7	9.2	34.1	42.3[c]	36.2	43.0	40.3
	23	0.5	A, B, E, G	77.6	72.2	74.3	10.0	9.9	42.1	44.2	41.8	37.1	36.8
July 13	2	0	A, J	37 030[b]	27 417[b,d]	30 100[b,d]	19.0	10.0[d]	33.5	38.9[c]	32.2	37.8	33.1[c]
	2	3	A, J	82 389[b]	62 724[b,d]	64 388[b,d]	24.6	10.7[d]	27.6	39.0[d]	28.7	39.3	34.3[c]
Aug. 3	2	1	E, J, N	11.3	8.0[c]	9.9	33.5	21.2[d]	33.3	35.4	34.0	24.3	26.1
	2	5	C, N	51.4	52.5	38.8[d]	7.3	6.6[c]	27.8	33.8[c]	31.3	55.1	49.7
Sept. 15	2	2	A, D, E, F	32 944[b]	32 907[b]	—	11.3	9.2	40.9	47.0	—	—	—

[a]Numerically dominant phytoplankton genera: A, *Microcystis*; B, *Aphanothece*; C, *Coelosphaerium*; D, *Lyngbya*; E, *Oscillatoria*; F, *Raphidiopsis*; G, *Ankistrodesmus*; H, *Chlamydomonas*; I, *Gloeocystis*; J, *Scenedesmus*; K, *Cyclotella*; L, *Melosira*; M, *Stephanodiscus*; N, *Synedra*; O, *Chroomonas*; P, *Cryptomonas*.

[b]Productivity expressed in disintegrations per minute per hour of incubation per sample volume filtered.

[c]Differences between enriched and control samples statistically significant at $P < 0.05$.

[d]Differences between enriched and control samples statistically significant at $P < 0.001$.

suggesting that components of the downlake phytoplankton community were also P limited at this time.

The phytoplankton community in the downlake epilimnion of Normandy Lake became N deficient in the summer of 1983 as a result of its isolation from riverine nutrient inputs and from the relatively N-rich hypolimnion. High rates of lipid synthesis by downlake epilimnetic phytoplankton were symptomatic of this N deficiency during this period. Lipid accumulation has frequently been observed in N-deficient algal cultures (Spoehr and Milner 1949; Fogg 1959; Shifrin and Chisholm 1981). Lipid synthesis accounted for an increased proportion of the photosynthate under N limitation in cultures of *Navicula pelliculosa* (Fogg 1956) and *Botryococcus braunii* (Ohmori et al. 1984). In both of these studies, the high rates of lipid synthesis caused by N deficiency were greatly depressed by NH_4 additions, as we observed in Normandy Lake phytoplankton. To our knowledge, these results from Normandy Lake are the first time that a relationship between N deficiency and increased rates of lipid synthesis has been documented in nature.

During the summer period when N became limiting in Normandy Lake, three other factors (temperature, light, and the presence of cyanobacteria) were also changing and could be viewed as alternative explanations for the observed increases in phytoplankton lipid synthesis. In a larger data set of C metabolism patterns at P_{max} determined for a broad range of limnological conditions, we found that the relative and absolute rates of lipid synthesis were not significantly correlated with temperature ($r = -0.11$, $p > 0.26$ and $r = -0.01$, $p > 0.88$, respectively, $n = 96$). Within Normandy Lake during July and August, there were large longitudinal and vertical differences in lipid synthesis over small temperature differences. Previously, the highest measurements of lipid synthesis (18–20%) that we encountered were always associated with winter diatom assemblages and associated low temperature and light intensity.

Similarly, light appeared not to be the causative factor. The July and August incubations of samples from stations located along the longitudinal axis of the reservoir were all conducted at the same light intensity, but still showed large variations in lipid synthesis. A photosynthesis–light intensity experiment conducted on August 17 with a sample from the metalimnetic Chl *a* peak showed that, at saturating light intensities, lipid synthesis still accounted for only 10% of the total fixed C. Therefore, the significant decline of absolute and relative lipid synthesis rates in situ between the epilimnetic and metalimnetic samples was not the result of a decrease below a critical light intensity.

The summer dominance of cyanobacteria was also probably not responsible for the high lipid synthesis. Lipid synthesis accounted for 18 and 5% of the fixed C in July and September when cyanobacteria made up over 90% of the algal cells present. Conversely, on the sampling dates when lipid synthesis accounted for over 30% of the fixed C at P_{max} (June and early August), cyanobacteria made up 4 and 40% of the cells present. Collyer and Fogg (1955) and Canto de Loura et al. (1987) have shown that lipid accumulation is not characteristic of N-starved cyanobacteria; however, at least one exception to this generalization has also been reported (De Vasconcelos and Fay 1974). In a survey of 30 species, Shifrin and Chisholm (1981) reported that the lipid content of green algae consistently increased under N stress whereas this effect was less predictable in diatoms. Interestingly, in Normandy Lake, the fraction of Chlorophyta cells present was significantly correlated with the relative rates of lipid synthesis (at P_{max}) at station 2 ($r = 0.88$, $p < 0.01$, $n = 9$); however, in the absence of data on the specific contributions of the various algal groups to total community C fixation, this must remain a speculative relationship.

The depression of photosynthesis caused by enrichment with a limiting nutrient has been attributed to competition for photogenerated ATP and reductant between the metabolic processes of photosynthetic C fixation versus uptake and assimilation of the nutrient (Falkowski and Stone 1975; Thomas et al. 1976; Healey 1979; Lean and Pick 1981; Lean et al. 1982). However, the changes in C metabolism in response to NH_4 and PO_4 additions observed in this study suggest that the effects of

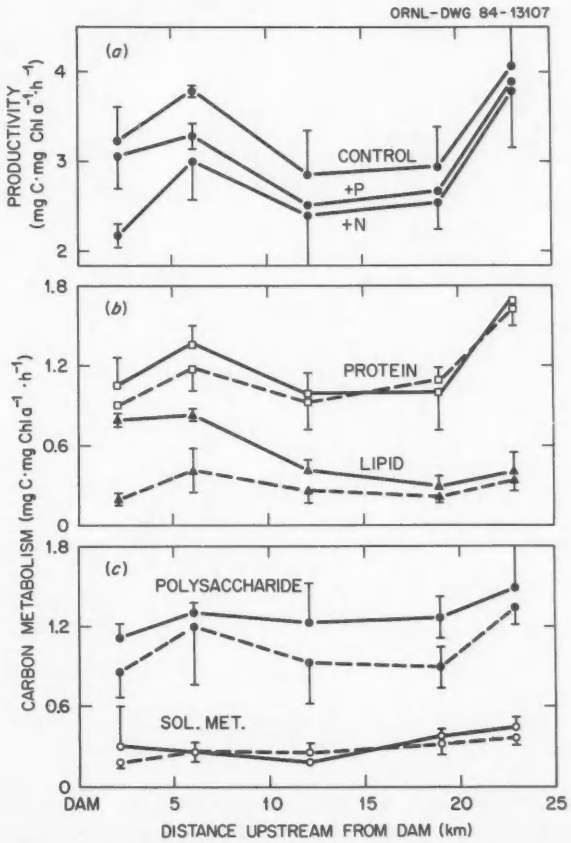

FIG. 6. Response to nutrient enrichment of near-surface phytoplankton assemblages collected from stations along the longitudinal axis of Normandy Lake on July 12, 1983. (a) Response of Chl *a* specific productivity to PO_4 and NH_4 enrichment; (b and c) response of photosynthetic C metabolism to unenriched (control, solid line) and NH_4-enriched (broken line) samples. Error bars represent 95% confidence intervals. Soluble metabolites = SOL. MET.

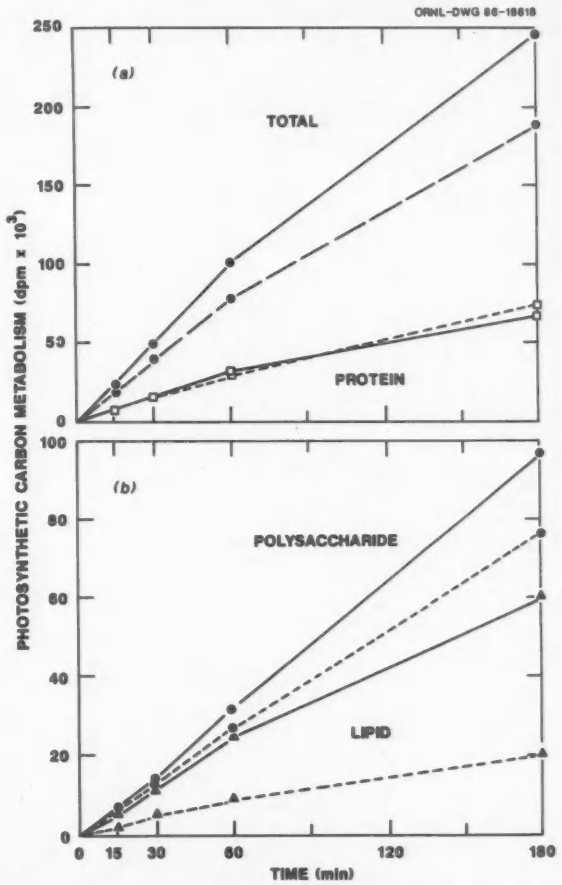

FIG. 7. Time course of photosynthetic C metabolism in an unenriched control and in NH_4-enriched 3-m samples from station 2 in Normandy Lake on July 13, 1983. (a) Total C uptake (●) and protein synthesis (□); (b) polysaccharide (●) and lipid (▲) synthesis. Solid line is the control and broken line is the NH_4-enriched treatment.

nutrient enrichment may be more complex than a mere intracellular reallocation of ATP and reductant. This should not be unexpected, as both NH_4 (Kanazawa et al. 1972) and inorganic phosphate (P_i) (Robinson and Walker 1981) play critical regulatory roles in photosynthetic C metabolism and biosynthesis.

In samples in which NH_4 inhibited photosynthetic C reduction, lipid synthesis was usually depressed, while protein synthesis was less sensitive and often showed a corresponding increase in relative importance. Protein and lipid synthesis may be viewed as competing pathways for photosynthate exported from the chloroplast to the mitochondria. This photosynthate may enter the tricarboxylic acid (TCA) cycle where C skeletons for amino acid and protein synthesis are produced or be converted to acetate for fatty acid and lipid synthesis (Stumpf 1984). Lipid synthesis in the cyanobacteria appears to function in a similar manner (Lem and Stumpf 1984). NH_4 enrichment stimulates C flow into the TCA cycle and amino acid synthesis in algae and cyanobacteria (Holm-Hansen et al. 1959; Hiller 1970; Kanazawa et al. 1972; Wegman and Pristava 1974; Lawrie et al. 1976; Ohmori et al. 1984). The reduction in lipid synthesis may therefore be caused by an efficient shunting of photosynthate into the TCA cycle at the expense of lipid synthesis. It has been established that some algae have two distinct enzyme systems for fatty acid synthesis (Ernst-Fonberg et al. 1974). Additionally, in a strain of *Chlorella*, a secondary pathway for lipid synthesis was rapidly induced under N limitation (Semenenko and Rudova 1976). This suggests that a distinct pathway for producing neutral storage lipids occurs in some algae during N deficiency, and it may be this alternative pathway that is particularly sensitive to NH_4 enrichment. Elrifi and Turpin (1986) and Birch et al. (1986) have recently reported that N additions to N-deficient *Selenastrum minutum* caused a decrease in photosynthesis by draining C intermediates from the photosynthetic C reduction cycle to supply a stimulated TCA cycle; this response is consistent with the data reported here. NH_4 can also decrease C fixation by uncoupling photophosphorylation from oxygen evolution (Krogmann et al. 1959).

Polysaccharide synthesis was depressed when photosynthesis was inhibited by PO_4 enrichment; however, this effect was less marked than the reduction in lipid synthesis caused by NH_4 additions. There are two well-known physiological mechanisms that may work together to cause a reduction in polysac-

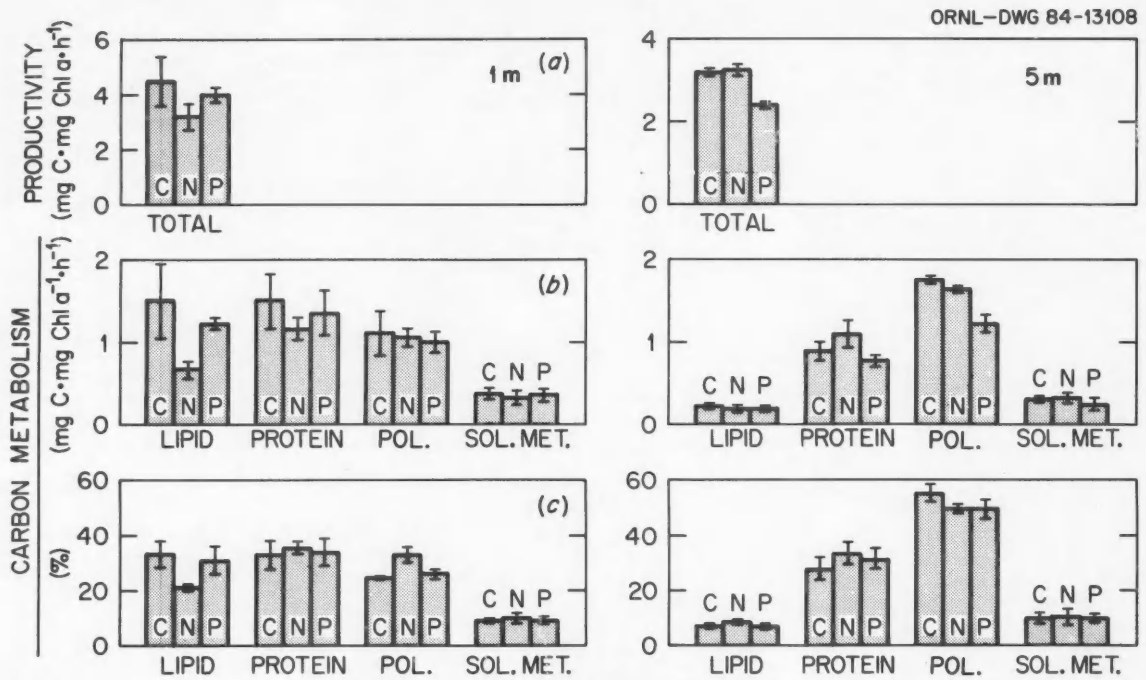

FIG. 8. Response of samples from 1 and 5 m from station 2 in Normandy Lake on August 3, 1983, to nutrient enrichment. (a) Chl *a* specific phytoplankton productivity; (b) absolute and (c) relative rates of photosynthesis C metabolism for control (C), NH_4-enriched (N), and PO_4-enriched (P) samples. Error bars represent 95% confidence intervals. Soluble metabolites = SOL. MET.; polysaccharides = POL.

charide synthesis. An intracellular increase in P_i can cause photosynthate to be drained from the chloroplast, the site of polysaccharide synthesis, through a triose phosphate-P_i translocator. This retards regeneration of intermediates within the photosynthetic C reduction cycle, thereby slowing the rate of photosynthesis (Heldt et al. 1977; Flugge et al. 1980; Usada and Edwards 1982). P_i also acts as an inhibitor of the enzymes involved in polysaccharide synthesis in algae and cyanobacteria (Preiss and Levi 1978; Preiss 1984).

We caution that some of the biochemical mechanisms resulting in reduced C fixation in association with nutrient enrichment have only been demonstrated at high concentrations of inorganic N or P. However, it is well established that nutrient-deficient phytoplankton often possess capabilities for enhanced nutrient uptake which can greatly increase intracellular concentration of that nutrient (McCarthy 1981; Lehman and Sandgren 1982) and exacerbate the regulatory effect of the nutrient on photosynthesis and C metabolism.

Our results establish a link between investigations of phytoplankton in nature and the results of numerous algal culture studies indicating that low N availability and/or low N cellular content leads to an increased cellular lipid content. Fogg (1956) reported that high rates of lipid synthesis in *Navicula* occurred only at moderate levels of cellular N deficiency. Under severe N deficiency, where lipids made up a high percentage of the cellular dry weight, lipid synthesis actually decreased in importance. While high rates of lipid synthesis may be common in moderately N-deficient conditions, the extreme N deficiency usually associated with high lipid accumulation in algal cultures may be rare in nature. An exception may be a naturally lipid-rich alga, such as *Botryococcus*, which has been reported to have a consistently high lipid content in nature (Aaronson et al. 1983). The occurrence of high rates of lipid synthesis, particularly when accompanied by an ability to depress lipid synthesis by NH_4 enrichment, appears to be a sensitive indicator of N deficiency in natural phytoplankton communities.

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Emergence of Chironomidae (Diptera) in Fertilized and Natural Lakes at Saqvaqjuac, N.W.T.

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Welch, H. E., J. K. Jorgenson, and M. F. Curtis. 1988. Emergence of Chironomidae (Diptera) in fertilized and natural lakes at Saqvaqjuac, N.W.T. Can. J. Fish. Aquat. Sci. 45: 731–737.

Chironomid emergence was quantified in four small lakes at Saqvaqjuac, N.W.T. (63°39′N), before and after lake fertilization. Emerging biomass responded immediately to increased phytoplankton production, reaching equilibrium the following year. Emergence from the reference lake was extremely variable, for no apparent reason. The emergence – phytoplankton production relationships found by Davies for the Experimental Lakes Area (~49°N) were generally valid for Saqvaqjuac lakes and Char Lake (74°42′), except that (1) biomass was better correlated than numbers because of increased mean size with increasing latitude and (2) total primary production was a better predictor than phytoplankton production alone because benthic photosynthesis increases with increasing latitude. Chironomid production seems to be a predictable function of total primary production throughout the latitudinal range of the small Canadian lakes examined.

On a quantifié le taux d'émergence de Chironomidés dans quatre petits lacs de Saqvaqjuac (T.N.-O.) (63°39′N) avant et après la fertilisation lacustre. La biomasse émergente a réagi immédiatement à l'augmentation de la production phytoplanctonique et a atteint un équilibre l'année suivante. Dans le lac témoin, le taux d'émergence était très variable pour aucune raison apparente. Les relations entre le taux d'émergence et la production phytoplanctonique établies par Davies dans le cas de la Région des lacs expérimentaux (~49°N) s'appliquaient généralement aux lacs Saqvaqjuac et Char (74°42′N) sauf que 1) la biomasse montrait une meilleure corrélation que l'abondance à cause d'une augmentation de la taille moyenne en fonction d'une augmentation de la latitude, et 2) la production primaire totale était une meilleure variable de prédiction que la production phytoplanctonique seule, étant donné que le taux de photosynthèse par les organismes benthiques augmente en fonction d'une augmentation de la latitude. La production de Chironomidés semble être une fonction extrapolable de la production primaire totale à toutes les latitudes des petits lacs canadiens étudiés.

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From 1977 to 1983, the Department of Fisheries and Oceans carried out a lake fertilization experiment at Saqvaqjuac, 36 km north of Chesterfield Inlet, N.W.T. (63°39′N). The objectives of the experiment were, first, to determine whether arctic lake phytoplankton production would respond to increased phosphorus and nitrogen supply as did temperate lakes (e.g. Experimental Lakes Area (ELA) (~49°N), northwestern Ontario; Schindler 1975) and, second, to quantify the response of lake secondary productivity to increased phytoplankton production resulting from fertilization. Chironomid emergence was chosen as one measure of secondary productivity because it is relatively easy to quantify, it probably constitutes a fairly constant proportion of chironomid production (Welch 1976), and it was thought to be a function of phytoplankton production (subsequently borne out by Davies 1980). Chironomid production is also a major part of the energy flow in arctic lakes and comprises a major part of the diet of lake-dwelling Arctic char (Welch 1973). Accordingly, chironomid emergence was monitored in the four primary study lakes. The present paper reports the results of those studies, along with its companion note (Welch et al. 1988) which explores some of the details of the emergence trapping technique.

Chironomid emergence has been studied at only a few arctic sites. Oliver (1968), Danks (1979), and Danks and Oliver (1972) discussed emergence phenology in the high arctic, where it was found that emergence by a given species occurred during a very narrow time window. Butler (1982a, 1982b) worked extensively on chironomid life cycles and emergence phenologies in tundra ponds at Barrow, Alaska. In those shallow ponds, which freeze to the bottom each winter, life cycles were prolonged up to 7 yr, even though emergence of each species occurred in a short period. Welch's (1973, 1976) study of chironomid emergence and energetics in Char Lake at Resolute, N.W.T. (74°42′N), and associated primary productivity studies (Kalff and Welch 1974; Welch and Kalff 1974) provide the only quantitative data on emergence and photosynthesis in an arctic lake containing fish, and as such is directly comparable with the Saqvaqjuac work reported here. Davies (1980) quantified Diptera (Chironomidae plus a few *Chaoborus*) emergence as a function of phytoplankton production in lakes at ELA in north-

western Ontario, and we have drawn heavily on that work for comparative purposes.

Methods

The study lakes lie in glacially scoured Precambrian granitic gneiss less than 3 km from the coast of Hudson Bay. Maximum winter ice thickness is typically 1.6–2+ m, followed by ice-out sometime between 28 June and 20 July. Maximum water temperature is 12–14°C and the small lakes do not premanently stratify. Freezeup occurs in late September. Nutrient loading is low and the lakes are oligotrophic. A more detailed site description can be found in Welch (1985). Information on nutrient loading, chemical limnology, and physical limnology is given in Welch and Legault (1986) and Welch et al. (1987). Details of the four experimental lakes and their treatments are given in Table 1.

We used submerged funnel traps (Davies 1984, fig. 6.4) equipped with a latex tubing bridle to serve as a shock absorber against wave action. The 60-cm-diameter (0.283-m^2) transparent cones had 250 mL glass collecting jars (46 mm I.D. necks) screwed into the tops. Polyethylene funnels with 6-mm foam neoprene collars, similar to the minicones of Rosenberg and Wiens (1983), were pressed inside the jar necks to minimize the sinking of dead insects out of the jars (Welch et al. 1988).

Traps were placed along four transects spaced equidistantly around each lake such that sampling effort was approximately proportional to the lake area at each depth (Table 1). Transects were reinstated at approximately the same locations each year. Traps at 0.5 and 1.0 m depth rested directly on the bottom rather than being suspended and were moved slightly after each sampling to avoid any long-term effects of traps resting permanently on the same spot. Traps were emptied twice weekly and the collected pupae and adults were preserved in 4% formaldehyde. At those water temperatures (4–12°C) there was little deterioration of specimens.

Because of limited manpower and a suspected large number of species, we decided to sort to size classes rather than to species, since we would still obtain emergence quantities even though interesting species-specific data would be lost. Each adult was sorted to sex, and the total thorax length viewed from the side was measured to the nearest 0.1 mm for specimens ≥1.0 mm thorax length and to the nearest 0.01 mm for smaller ones. Pupae were counted and were assumed to have the same size distribution as the adults in the same trap sample. Freshly emerged adults were collected, measured, dried at 105°C, and weighed to produce the weight–length relationship in Fig. 1.

To obtain lakewide annual emergence of adults and pupae, the annual emergence by size class and sex was first calculated for each sampling depth. Emergence per square meter at 0.5 m depth was multiplied times the 0.0–0.5 m stratum lake area, the mean of the 0.5 and 1.0 emergence per square meter was multiplied times the 0.5–1.0 m stratum area, and so on to maximum depth. The data were summed to give lakewide total emergence by size class. Biomass was computed by combining those data with the weight–length relationship in Fig. 1.

Results and Discussion

Patterns of Emergence

A total of 21 lake years of chironomid emergence data was obtained (Table 2). Emergence began 5–15 d after complete ice-out, but there was no relationship between ice-out data and the onset of emergence.

The depth distribution of emergence in each lake was highly variable from year to year. For example, maximum emergence per square meter in P + N Lake occurred at 0.5 m one year and at 10 m the following year. Total lakewide emergence, however, was always dominated by shallower depths because of the influence of lake morphometry.

Size–frequency distributions of emerged adults showed no consistent trends with time (within year and by year) or depth, with the exception that mean adult size tended to increase in P + N Lake after fertilization and probably did so in Jade Lake as well, since emerging adults in 1979 (the first year of fertilization) were smaller than in subsequent years (Table 2). For almost every lake year, a consistent abundance peak at 0.9 mm thorax length was found, and this was later identified as being composed of *Corynoneura* spp., mostly *C. scutellata* Winn. Abundance peaks at other length frequencies were not as consistent year to year and lake to lake, no doubt caused by changing species composition. However, it appeared that only a relatively few species dominated the emergence in all lakes, and in retrospect it might have been as fast to sort the catch to the most common species, perhaps leaving the rarer species to be analyzed on basis of size. This would have allowed a more thorough interpretation of the phenology and depth distribution of emergence at Saqvaqjuac. (Should the reader wish to pursue the analysis further, all catches are archived and available from the authors.)

Comparison of Total Emergence with Annual Primary Production

The quantity of prefertilization emergence was similar in P + N and Far lakes (Fig. 2), as was phytoplankton production

TABLE 1. Nutrient status and emergence trapping intensity for four lakes at Saqvaqjuac, N.W.T.

Lake	Area (ha)	Z_{max} (m)	Experimental treatment	Period trapped[a]	Trap depth (m) (no. of traps at each depth in parentheses)
P + N	7.09	10.2	0.1 g P, 1.0 g N·m^{-2} added 1979–83	1977–83	0.5, 1, 2, 3 (4); 4, 5, 6, 7 (2); 8, 9, 10 (1); total = 27 traps
Far	3.70	8.9	0.1 g P·m^{-2} added 1979–83	1977–83	0.5, 1, 2, 3 (4); 4, 5, 6, 7, 8 (2); total = 26 traps
Spring	6.90	7.1	Control	1978–83	0.5, 1, 2, 3 (4); 4, 5, 6, 7 (2); total = 24 traps
Jade	3.63	5.2	0.2 g P, 2.0 g N·m^{-2} added 1979–80	1979–83	0.5, 1, 2, 3 (4); 3 (3); 5 (1); total = 20 traps

[a]No trapping in 1982.

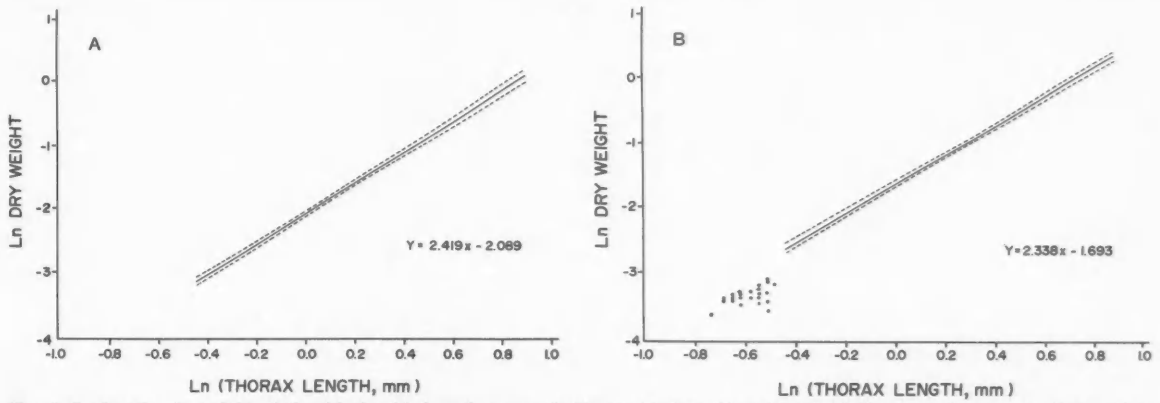

FIG. 1. Ln length – ln weight relationship for dried newly emerged chironomid adult (A) males and (B) females at Saqvaqjuac. Broken lines indicate the 95% confidence band for the solid line. Females <0.62 mm thorax length fell into a separate group and were treated separately.

TABLE 2. Summary of annual totals of chironomid emergence and primary production in Saqvaqjuac lakes and selected means.

Lake	Year	Nutrient addition	Phytoplankton production ($gC \cdot m^{-2} \cdot yr^{-1}$)[a]		Number emerging ($no. \cdot m^{-2} \cdot yr^{-1}$)		Biomass emerging ($g \cdot m^{-2} \cdot yr^{-1}$)		Mean wt per adult (mg)	
P + N	1977	None	4.7	3.8	737	743	0.137	0.126	0.186	0.170
	1978	None	2.9		749		0.114		0.153	
	1979	P + N	9.8	14.7	1839	1921	0.238	0.421	0.130	0.218
	1980	P + N	12.9		1982		0.524		0.265	
	1981	P + N	21.4		1942		0.502		0.258	
	1983	P + N	—		1931		0.487		0.252	
Far	1977	None	2.6		1007		0.251		0.249	
	1978	None	3.6		464		0.075		0.163	
	1979	P	2.5	3.2	811	1108	0.130	0.269	0.160	0.223
	1980	P	2.5		1304		0.347		0.266	
	1981	P	5.0		1953		0.543		0.278	
	1983	P	—		1182		0.241		0.204	
Spring	1978	None	—		436		0.100		0.230	
	1979	None	4.7		830		0.204		0.246	
	1980	None	8.0	7.1	857	1632	0.269	0.419	0.314	0.268
	1981	None	8.5		3208		0.784		0.244	
	1983	None	—		7689		1.286		0.167	
Jade	1979	P + N	4.4		1078		0.195		0.181	
	1980	P + N	9.5	6.2	1749	1224	0.462	0.308	0.264	0.254
	1981	None	4.6		845		0.267		0.316	
	1983	None	—		1263		0.457		0.362	
								$\bar{x}$ =	0.233	

[a]July–August openwater season.

(Table 2). Upon fertilization of P + N Lake in 1979, there was an increase in small chironomid adults late in the same season. Emergence (both numbers and biomass) in 1980, 1981, and 1983 was constant at four times the prefertilization level of the previous 2 yr and was proportional to the increase in phytoplankton production (Table 2). Far Lake did not show a consistent increase in emergence after fertilization with P only, although the data were more variable than for P + N Lake (Fig. 2). Far Lake phytoplankton did not increase after fertilization (Table 2).

We had no prefertilization data for Jade Lake. The lowest emergence was in 1979, the first year of fertilization (Fig. 3). As a result of fertilization and concomitant increased primary production and increased lake respiration, winter oxygen was very low early in 1981 (maximum concentrations <2 mg $O_2 \cdot L^{-1}$). The lake was anoxic below 2 m (Welch and Bergmann 1985) and therefore was not fertilized in subsequent years. These conditions may have reduced the number of chironomids emerging in 1981. Although respiration increased in P + N Lake as a result of fertilization, the lake did not become completely anoxic (Welch and Bergmann 1985), and we doubt if the chironomid population was reduced very much due to anoxia in the deep water.

The annual biomass emerging in P + N, Far, and Jade lakes generally met our expectations that any changes in phytoplankton production would result in corresponding changes in emer-

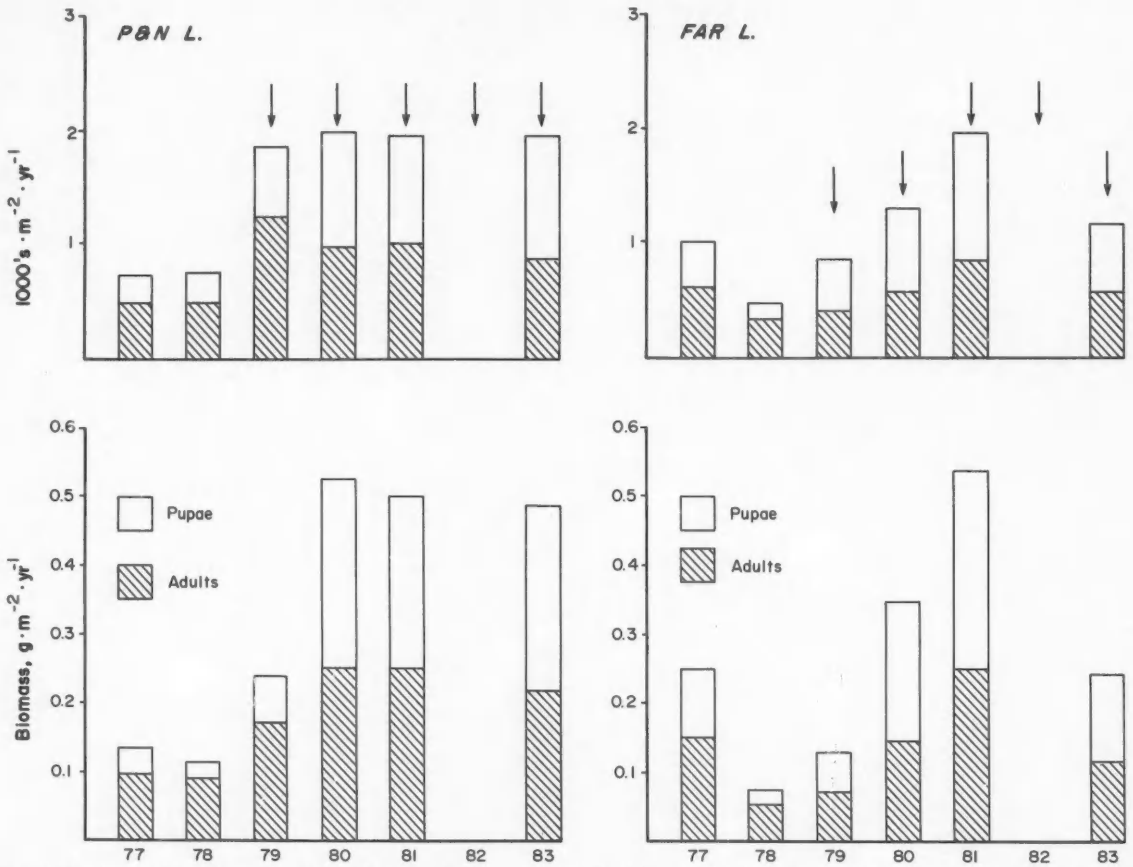

FIG. 2. Numbers and biomass of emerging Chironomidae in P + N and Far lakes, 1977–83. Arrows indicate years in which the lakes were fertilized (P only in Far Lake, P and N in P + N Lake).

gence (Davies 1980). However, Spring Lake, originally chosen as a reference system for the nutrient experiments, exhibited a dramatically different trend over the same period. Although phytoplankton production remained quite constant in Spring Lake (Table 2), emergence increased steadily each year; by 1983, windrows of dead insects (largely *Corynoneura scutellata*) could be scooped from the lake surface, a phenomenon not observed in any of the other numerous lakes in the area. There were no obvious correlates to account for the 13-fold increase in emerging biomass (Table 2), but it is worth noting that Spring Lake arose from the sea only about 350 yr ago and still appears to be in a period of chemical and biological instability (Welch and Legault 1986). We expect that the Spring Lake results define the limits of variability for lakewide emergence at Saqvaqjuac and that annual variability for nearly all other lakes in the area is much lower.

In Fig. 4 the relationships between emergence and primary production for Saqvaqjuac lakes are compared with those found by Davies (1980) for ELA lakes. The ELA relationships overestimate numbers of emerging insects and underestimate biomass at Saqvaqjuac, suggesting that Saqvaqjuac chironomids are heavier than those studied by Davies. This is verified by Fig. 5, which includes Char Lake. Although there is a wide range of adult weights in a given lake, depending on the species composition, it appears that the mean species weight is higher in the arctic, e.g. the adult dry weights of the six common species in Char Lake range from 0.12 to 1.16 mg (calculated from Welch 1973, table 2). Note also that there is no relationship between primary production and mean adult size in the Saqvaqjuac lakes, unlike for ELA (Fig. 5; Table 2).

Numbers of chironomids emerging at Saqvaqjuac are fairly well predicted by the ELA relationship relating numbers to summer openwater phytoplankton production (Fig. 4A); recalculation of the regression using both data sets would decrease the importance of the Lake 223 outlier and increase the correlation with the Saqvaqjuac and Char Lake points. The biomass of emerging chironomids for arctic lakes is poorly predicted by the ELA relationship based only on summer openwater phytoplankton production (Fig. 4B). However, if total primary production is used as the independent variable, the arctic lakes are predicted much more closely by the ELA relationship. This is reasonable if we assume that emergence ultimately reflects the total energy available in a lake (see discussion below). At ELA, summer phytoplankton account for nearly all photosynthesis; benthic primary production is only a few percent of total photosynthesis (Schindler et al. 1973) and winter photosynthesis is negligible (E. Fee, Freshwater Institute, Winnipeg, Man., pers. comm.). But at Saqvaqjuac, about half the total phytoplankton

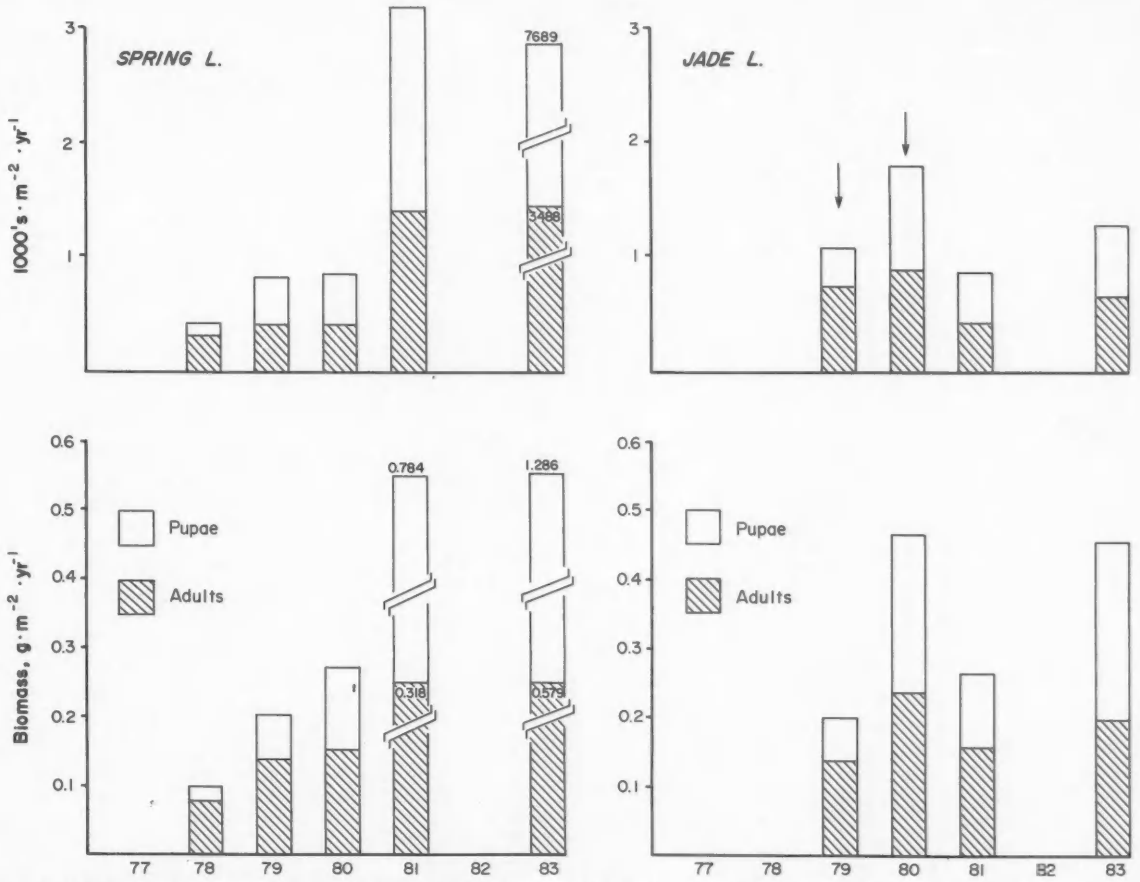

FIG. 3. Numbers and biomass of emerging Chironomidae in Spring and Jade lakes, 1977–83. Arrows indicate years in which Jade Lake was fertilized with P and N.

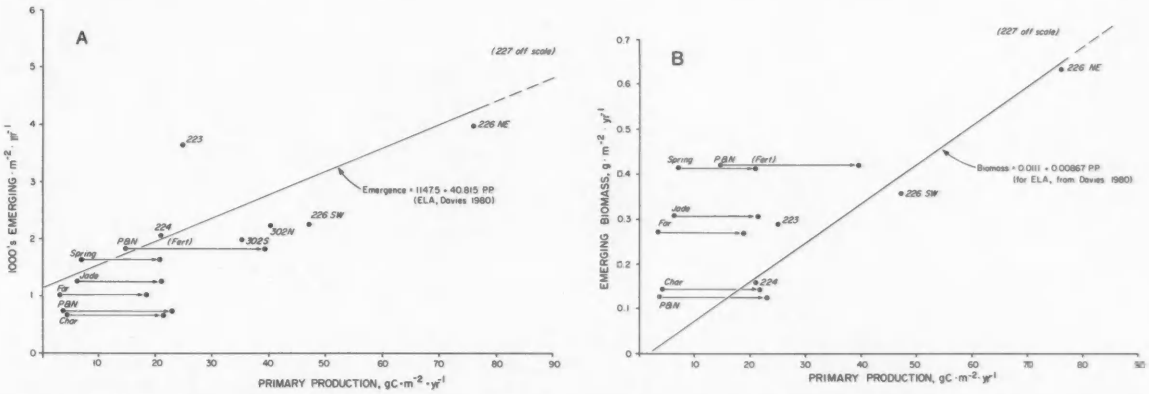

FIG. 4. Mean (A) numbers and (B) biomass of emerging chironomids as a function of primary production, 1977–83. Dots represent summer openwater phytoplankton production only. Arrows link summer phytoplankton production (lower value) and total annual primary production, planktonic and benthic (higher value).

production occurs beneath the ice (H. E. Welch et al., unpubl.), and benthic primary production is about equal to the phytoplankton (H. E. Welch, and M. A. Bergmann, unpubl.). Macrophyte production is negligible in both sets of lakes (although it is important in Char Lake). Assuming that biomass is a better measure of productivity than numbers emerging, we expect that relationships between emerging biomass and total production in different geographic regions will be stronger than

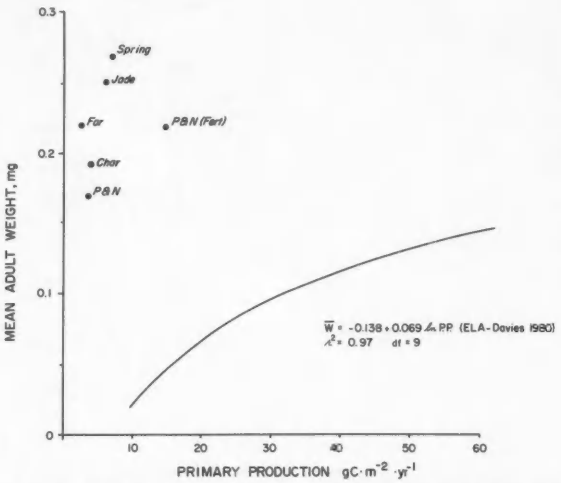

FIG. 5. Mean adult chironomid weight as a function of openwater phytoplankton production for Saqvaqjuac and the Experimental Lakes Area.

correlations between numbers emerging and various fractions of lake primary production.

The hypothesis that chironomid emergence is related in some predictable way to primary production involves a number of assumptions, as follows. The total energy degraded through the food chain must equal total autochthonous production, ignoring import, export, and storage. The type of plant material produced, whether it be phytoplankton, benthic algae, or macrophytes, may also determine the nature and efficiency of secondary productivity. Benthos accounts for a larger portion of energy flow in small lakes, and chironomids are usually a major component of the benthos. Emergence seems to constitute a fairly constant fraction of benthic dipteran production (Simuliidae, Speir and Anderson 1974; chironomids, Welch 1973). If there were no mortality, essentially all production would emerge, but a variable and usually high proportion of fourth instar larvae do not appear in emergence traps (Davies 1984, table 6.3), and it is likely that predation accounts for the difference. Predation on fourth instar larvae and pupae by Arctic char is high in Char Lake (Welch 1976) and in the four Saqvaqjuac lakes (unpubl.), and lake trout, whitefish, and other fish species are common in the ELA lakes where Davies conducted his studies. Unfortunately, none of these three studies quantified the effects of fish predation on potential dipteran emergence.

Thus there are many factors which combine to give a particular yield of chironomid emergence compared with primary production in a given lake. Analysis of early IBP data showed only a weak correlation between macrobenthos production and primary production (Morgan 1980, fig. 6.7); interestingly, the six data points for arctic and alpine lakes tend to show relatively high macrobenthos production per unit primary production, which is also found for Saqvaqjuac and Char lakes (Fig. 4B). Analyzing the same data set, Brylinsky (1980, table 9.10) found a weak correlation between herbivorous benthos, carnivorous benthos, and phytoplankton production. These weak correlations are understandable given the very diverse water bodies and techniques used to gather the IBP data set. The ELA and Saqvaqjuac data sets are very comparable in that the methods used to estimate emergence and primary production, as well as the geological setting, are similar, although the climates are different. The ratio of emergence biomass to total primary production at ELA is 0.0087 g·g C^{-1} (Davies 1980; Fig. 5) whereas the mean is about 50% higher, 0.0129 g·g C^{-1}, for the 16 Saqvaqjuac lakes years with both emergence and primary production data (Table 2) and lower, 0.0061 g·g C^{-1}, in Char Lake (0.1315 mg·m^{-2} emergence and 21.1 g C production, Welch and Kalff 1974). Thus, it appears that chironomid emergence is a fairly constant proportion, about 1%, of primary production throughout the latitudinal range of small oligotrophic Canadian lakes, and the relative importance of chironomids in those food chains, therefore, is probably also constant.

Chironomid emergence is perhaps the easiest and fastest quantitative measure of secondary production currently available for lakes, although it is still difficult to quantify in absolute terms. The development of submerged, integrating emergence traps which can be left for months at a time would greatly simplify the use of emergence as a tool for the assessment of secondary production (Welch et al. 1988).

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NOTES

Measuring Abundance of Emerging Chironomidae (Diptera): Experiments on Trap Size and Design, Set Duration, and Transparency

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Welch, H. E., J. K. Jorgenson, and M. F. Curtis. 1988. Measuring abundance of emerging Chironomidae (Diptera): experiments on trap size and design, set duration, and transparency. Can. J. Fish. Aquat. Sci. 45: 738–741.

Experiments at Saqvaqjuac, N.W.T. (63°39′N) showed that submerged funnel traps (0.283 m^2) had equal catch rates at 10–12°C, independent of emptying interval between 1 and 6 d. Inverted funnels in the trap jars reduced the loss of active pupae, and probably sinking dead insects. Catch rates were independent of trap size (0.071–0.283 m^2) at emergence densities $\leq 418 \cdot m^{-2} \cdot d^{-1}$. Preserving-type traps which retained the catch had the same daily catch rate as regular traps when left in place up to 18 d, but none of the preservatives tried was satisfactory and decay was a problem. Catch rates for clear and opaque traps were similar.

Des expériences réalisées à Saqvaqjuac (T.N.-O.) (63°39′N) ont montré que des pièges à entonnoir immergés (0,283 m^2) présentaient des taux de capture égaux à 10–12°C, indépendamment de l'intervalle (1 à 6 jours) du vidage des pièges. La présence d'entonnoirs renversés dans les bocaux servant de pièges a permis de réduire la perte de pupes actives et aussi, probablement, celle des insectes morts en train de couler. Les taux de capture étaient indépendants de la taille des pièges (0,071–0,283 m^2) aux densités d'émergence de ≤ 418 $m^2 \cdot d^{-1}$. Les pièges à produit de conservation qui retenaient les animaux capturés présentaient le même taux de capture quotidien que les pièges standard lorsqu'ils étaient laissés en place pendant une période pouvant atteindre 18 d, mais aucun des produits de conservation utilisés ne s'est avéré satisfaisant et il y a eu décomposition des spécimens. Les taux de capture des pièges transparents ou opaques étaient semblables.

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Saqvaqjuac is a Department of Fisheries and Oceans research station 36 km north of Chesterfield Inlet on the northwest coast of Hudson Bay (63°39′N) and was the site of a whole-lake fertilization experiment conducted during 1977–83. Chironomid emergence was routinely monitored in several lakes as a measure of secondary production. The experiments reported here were performed on P + N Lake, a 7.1-ha, 10-m-deep oligotrophic lake which received an added 1 g N and 0.1 g $P \cdot m^{-2} \cdot yr^{-1}$ during July–August 1979–83. Site background information is given in Welch (1985). Water temperature during these experiments was 10–12°C.

In conjuction with the ongoing trapping program for emerging chironomid adults (Welch et al. 1988), two experiments (reported in this note) were performed to test certain assumptions about trapping efficiency. The first (1980) experiment tested the effects of trap set duration on catch and the catch efficiency of a preserving-type trap; the second (1981) experiment tested the effects of trap size, the transparency of traps, and the presence or absence of a funnel in the collecting jar on trapping efficiency.

Methods

The basic traps were the submerged funnel traps described in Davies (1984, fig. 6.4) with shock-absorbing bridle. The 60-cm-diameter (0.283-m^2) transparent cones had 250-mL glass collecting jars (46 mm I.D. necks) screwed into the tops. To minimize the sinking of dead insects out of the jars, polyethylene funnels with 6-mm foam neoprene collars were pressed inside the jar necks (similar to the minicones of Rosenberg and Wiens 1983). The funnel stems were cut off and oval openings ~7 × 4 mm ground into the apices.

In addition to these regular-format traps, we used similar traps without the jar funnel (to determine if the funnel inserts reduced loss), half- and quarter-sized traps (to determine if there were size effects on trapping efficiency), and opaque traps (painted externally with flat black paint). Unless otherwise noted, traps were emptied twice weekly (3- to 4-d intervals). Traps were not cleaned during the trapping season; the slight growth of periphyton on the traps was only faintly visible.

We also wanted to develop a "preserving trap" which would collect and preserve emerging chironomids for weeks or months

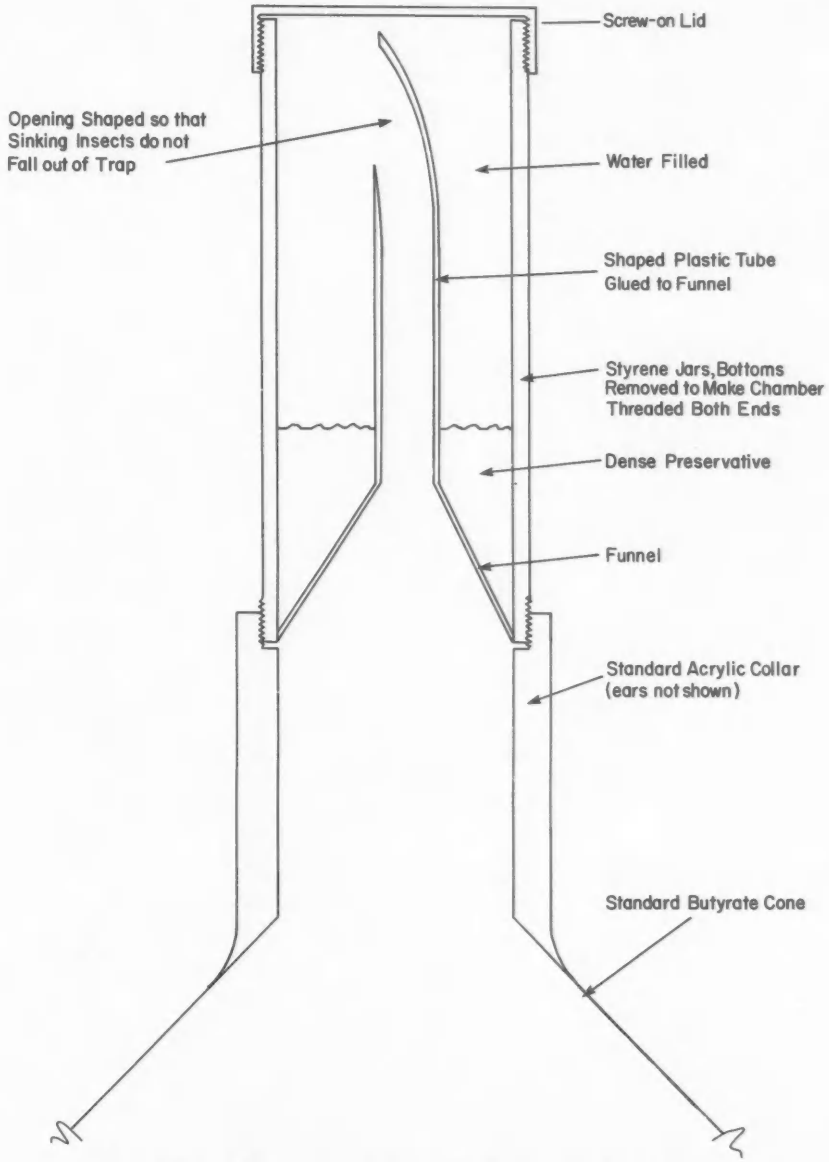
Screw-on Lid
Opening Shaped so that Sinking Insects do not Fall out of Trap
Water Filled
Shaped Plastic Tube Glued to Funnel
Styrene Jars, Bottoms Removed to Make Chamber Threaded Both Ends
Dense Preservative
Funnel
Standard Acrylic Collar (ears not shown)
Standard Butyrate Cone

FIG. 1. Preserving-type trap used on 60-cm-diameter submerged funnel trap.

at a time. For this purpose, we modified the regular traps by replacing the glass jars with a transparent styrene cylinder containing a long-stemmed funnel glued inside (Fig. 1). Preservative liquids were placed inside the styrene cylinders. Although we tried diesel fuel, salt water and formalin, and Mistovan®, phenoxyethanol was the preservative used routinely.

Traps were suspended in lines of five, with an anchor at the end of each line and float between each trap (similar to Davies 1984, fig. 6.5c). In the 1980 experiment, four lines of five traps each (a 4 × 5 grid) were placed in an area of uniform depth (2.8 m) and fine sediment substrate. Lines were ~6 m apart and traps were 1.0 m apart on each line. For the 1981 experiment, five lines of five traps each (a 5 × 5 grid) were placed in the same location as in the 1980 experiment. Deployment in both years was for the length of the emergence season, approximately mid-July to late August.

In 1980 there were 10 treatment types, two replicates of each, to test the effects of different trap emptying intervals for regular and preserving traps (Table 1). In 1981 there were five treatment types, five replicates of each, to test the effects of jar funnel absence, trap size, and transparency (Table 2).

We performed two-way ANOVA on the 1980 results to test for position effects. We performed three-way ANOVA (for a Latin-square design) on the 1981 results to test for position and trap-type effects. One-way ANOVA was used to compare further the effects of trap type in 1980 and 1981.

TABLE 1. Experimental randomized design for emergence trap grid, 1980. Treatments: 1 = regular trap, emptied every 1 d; 2 = regular trap, emptied every 2 d; 3 = regular trap, emptied every 3 d; 4 = regular trap, emptied every 4 d; 5 = regular trap, emptied every 5 d; 6 = regular trap, emptied every 6 d; 7 = preserving trap, emptied every 3 d; 8 = preserving trap, emptied every 6 d; 9 = preserving trap, emptied every 12 d; 10 = preserving trap, emptied every 18 d.

	Treatment No. (column)				
Trapline (row)	1	2	3	4	5
A	10	2	3	8	5
B	2	8	3	1	10
C	7	4	7	1	6
D	5	9	4	9	6

TABLE 2. Experimental Latin-square design for emergence trap grid, 1981. Treatments: 1 = regular trap (0.283 m²); 2 = regular trap, without jar funnel; 3 = regular trap, opaque; 4 = regular trap, half-sized (0.141 m²); 5 = regular trap, quarter-sized (0.071 m²).

	Treatment No. (column)				
Trapline (row)	1	2	3	4	5
A	1	2	3	4	5
B	5	1	2	3	4
C	4	5	1	2	3
D	3	4	5	1	2
E	2	3	4	5	1

TABLE 3. Total chironomid emergence for each trap in 1980.

	Total number of chironomids trapped (column)				
Trapline (row)	1	2	3	4	5
A	600	553	525	468	354
B	621	680	569	447	850
C	562	449	450	539	520
D	520	780	646	—[a]	620

ANOVA Table: sum of adults and pupae

Source of variation	df	Sum of squares	Mean square	F	PR > F
Row	3	0.2453	0.0818	2.16	0.146
Column	4	0.0734	0.0184	0.48	0.747
Error	12	0.4547	0.0379		
Total	19	0.7734			

[a]Missing data point was estimated according to Sokal and Rohlf (1980).

Results

1980 experiment — The total chironomid catch (emerged adults plus pupae) showed no position effect (Table 3; $p = 0.15$ (rows), $p = 0.75$ (columns), two-way ANOVA), indicating that emergence density was uniform beneath the experimental grid. One-way ANOVA also showed that there was no significant effect of trap-emptying interval for regular or preserving-type traps. The proportion of pupae was very high in the preserving traps but it was not assessed precisely because adults and pupae could not always be differentiated due to decay.

1981 experiment — Results are summarized in Table 4, from which we conclude the following:

TABLE 4. Total chironomid catch by the five traps in each treatment in 1981.

Treatment	No. of adults	No. of pupae	Total	% pupae
Regular	5853	6773	12 626	54
No funnel	6233	1118	7 351	15
Opaque	2455	9087[c]	11 542	79
Half-sized[a]	8282	6624	14 906	44
Quarter-sized[b]	8476	3468	11 944	29

ANOVA Table: sum of adults and pupae

Source of variation	df	Sum of squares	Mean square	F	PR > F
Row	4	8 792	2198.0	2.33	0.115
Column	4	1 458	364.5	0.39	0.814
Trap type	4	12 031	3007.8	3.19	0.053
Error	12	11 301	941.7	1.97	
Total	24	33 582			

[a]Half-sized regular-format traps; catch multiplied by 2.
[b]Quarter-sized regular-format; catch multiplied by 4.
[c]Missing data point was estimated according to Steel and Torrie (1980, p. 227).

(1) There was no detectable position effect for total emergence ($p = 0.12$ (rows), $p = 0.81$ columns), indicating that emergence density was uniform beneath the experimental grid.

(2) One-way ANOVA indicated that traps without funnels caught fewer pupae than regular traps ($p < 0.001$). The total chironomid catch of traps without funnels was low although the difference was not very significant ($p = 0.053$).

(3) Based on Latin-square analysis by ANOVA with rows, columns, and trap type as treatments, we detected no differences between opaque traps and regular traps ($p = 0.6$ for total emergence), but a higher fraction did not eclose (79%) as compared with regular traps (54%; difference significant at $p \leq 0.001$, one-way ANOVA).

(4) The half- and quarter-sized trap catches were proportional to those of regular traps ($p = 0.6$, one-way ANOVA).

(5) There was a significant ($p < 0.001$, one-way ANOVA) increase in pupae to adult ratios with decreasing trap size.

Discussion

Effects of funnels in the trap jars — Traps without funnels caught only 17% as many pupae as did regular traps, and the total catch was only 58% of regular traps whereas the adult catch was nearly the same (Table 4). This suggests that there was selective escape of active pupae, many of which are capable of swimming downward at least 1 m despite an often obvious gas layer visible between the pupal skin and the enclosed adult fly (H. E. Welch, pers. obs.). The funnels also retained dead insects that sank inside the jars, a considerable portion of the catch after several days, although our experimental results cannot be used to assess the value of funnels for the retention of dead chironomids. Rosenberg and Wiens (1983) tested the effects of funnel inserts (minicones) at South Indian Lake (57°N) and found that traps fitted with minicones usually caught less than those without minicones, although the differences were not significant. However, there the minicones retained invertebrate predators such as *Leptodora* spp. and water mites, which consumed part of the catch (Rosenberg and Wiens 1983), and traps were emptied after 48 h, so the loss of dead insects should have been small in any case. In Saqvaqjuac lakes which contain

Arctic char (*Salvelinus alpinus*) or lake trout (*S. namaycush*, our emergence traps with funnels do not retain invertebrate predators.

Effects of trap size — Since total catch was independent of trap size, the half-sized (0.142-m^2) trap could have been used for our regular sampling program (Welch et al. 1988), thereby reducing handling problems. A 0.1-m^2 trap would have captured about 175 insects in 1980 (Table 1), and less at certain locations in other Saqvaqjuac lakes (unpubl.), thereby resulting in unacceptably high variance. Conversely, as total catch (= trap size) increased (Table 4), so did the proportion of pupae; apparently, pupae tended to delay eclosion as crowding increased. This particular "trap saturation" effect (Davies 1984) occurred at relatively low densities ($\leq$120 insects per trap emptying), although it was not a problem during our routine trapping program, since both pupae and adults were retained by the funnels and pupae served the same purpose as adults (Welch et al. 1988).

Trap transparency — Although we found no statistical difference in total catch between opaque and transparent traps (Table 4), several investigators have found a strong correlation between trap transparency and catch, with reductions ranging from 35 to 90% for opaque traps (reviewed in Davies 1984, fig. 6.14). Aside from the extended daylight period at Saqvaqjuac, we can see no obvious reasons why our results should be different from those at lower latitudes. We did find that a higher proportion remained as pupae in opaque traps (Table 4), suggesting that pupae in darkened traps found conditions less ideal for emerging than when they were in transparent traps, and delayed eclosion. These results suggest that the light coating of periphyton which collected on regular traps during routine trapping did not reduce the total catch.

Preserving traps — In preserving traps, there was only a very small air bubble in the preserving chamber, and very little (<5%) of the total catch was in the adult form. Pupae entering such traps died before eclosion but were not able to escape from the trap. It is encouraging that the preserving traps caught at least as many insects as the regular traps, suggesting that few if any ascending pupae detected the preservatives in the traps and were able to descend back down and out of the trap. The main problem with the preserving traps was that none of the preservatives worked well enough to prevent decay, to the point where pupae could not always be distinguished from adults in the 12- and 18-d traps. The use of formalin held in a porous reservoir may solve this problem (M. G. Butler, Department of Zoology, North Dakota State University, Fargo, ND, pers. comm.).

Development of a preserving trap that could be left in place for long periods would be valuable. A major cost associated with monitoring insect emergence comes from the labor of emptying traps. Although the temporal distribution of emergence is lost, for many purposes, integrating traps that can be left in place for an entire season would be ideal. Davies (1980) has shown that chironomid emergence is a function of primary production, which is not surprising, since emergence is a large and fairly constant proportion of chironomid production (Welch 1967, 1976) and chironomid production is closely linked to the energy flow within a lake. The emerging biomass from preserving traps deployed at the beginning of the ice-free season and emptied in the fall could be dried and weighed, giving a relatively cheap measure of secondary production.

Surface traps that capture and preserve flying adults are easy to design (e.g. Welch 1967) although they are susceptible to disturbance by people, wind, and waves. Submerged traps are largely immune to such effects, but provision must be made to retain pupae as well as adults.

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Vertical Distribution of Sediment and Organic Debris in Coho Salmon (*Oncorhynchus kisutch*) Redds in Three Small Oregon Streams[1]

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Vertical distribution of sediment (particle diameter <3.33 mm) and organic debris was studied in coho salmon (*Oncorhynchus kisutch*) redds in three Oregon coastal streams subjected to different degrees of logging. Gravel composition in frozen cores varied significantly among redds in both logged streams. Sediment content in redds appeared to decrease with depth in the logged streams, but differences in gravel composition among streams obscured differences among depths. Sediment layers of variable thickness were visible in several core samples. In one logged stream, redds contained an average of 25% less sediment than redds used 1 yr earlier. Organic content of the gravel represented <2.8% of the core samples by weight and was directly related to the quantity of sediment in the streambed. The pattern of vertical distribution of organic debris in the clearcut stream (most abundant near the surface) differed significantly from that in the other two streams. Patterns of distribution and variability in gravel composition were consistent with post-logging changes in gravel permeability and survival to emergence of coho salmon.

Les auteurs ont étudié la distribution verticale de particules sédimentaires (diamètre < 3,33 mm) et de débris organiques dans des nids de saumons coho (*Oncorhynchus kisutch*) de trois cours d'eau côtiers de l'Oregon situés dans des zones d'exploitation forestière d'intensité variable. La composition du gravier de carottes congelées variait de façon significative entre les nids des deux cours d'eau des zones exploitées. La teneur en sédiments des nids semblait décroître avec la profondeur dans les cours d'eau exploités, mais les écarts de composition du gravier entre les cours d'eau avaient pour effet d'obscurcir les écarts entre les profondeurs. On pouvait voir des couches sédimentaires d'épaisseur variable dans plusieurs des carottes. Dans l'un des cours d'eau des zones exploitées, les nids contenaient en moyenne 25 % de moins de sédiments que ceux qui avaient été utilisés l'année précédente. La teneur en matière organique du gravier représentait moins de 2,8 % du poids des carottes échantillons et présentait une relation directe avec la quantité de sédiments dans le lit du cours d'eau. L'allure de la distribution verticlae des débris organiques dans le cours d'eau de la zone ayant subi une coupe à blanc (plus abondants près de la surface) différait de façon significative de celle notée dans les deux autres cours d'eau. L'allure de la distribution et de la variabilité de la composition du gravier après la coupe correspondait aux modifications de la perméabilité du gravier et du taux de survie à l'émergence des saumons coho.

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Logging and associated activities in small watersheds of the Pacific Northwest have often resulted in increased levels of suspended and deposited sediment in streams (e.g. Burns 1970, 1972; Brown and Krygier 1971; Beschta 1978; Adams and Beschta 1980). Short-term pulses of suspended sediment have potentially detrimental effects on behaviors of juvenile salmonids (Berg and Northcote 1985). Deposition of inorganic sediment in spawning beds represents a longer-term phenomenon related to survival to emergence of salmonid fishes, with survival a decreasing function of the quantity of fine sediment (Phillips et al. 1975; Lotspeich and Everest 1981). Documented effects include suffocation of fish embryos or larvae (Harrison 1923; Cordone and Kelley 1961) and entrapment of larvae within the gravel matrix (Koski 1966; Dill and Northcote 1970; Phillips et al. 1975). Increased deposition of fine organic debris associated with logging may be important in determining the oxygen demand of intragravel water (McNeil and Ahnell 1964; McNeil 1966). Relatively few studies of sediment have been accomplished in natural environments, and knowledge of relationships between fine sediment and reproductive success of salmonid fishes remains rudimentary (Everest et al. 1987).

During clearcut logging along a headwater stream in Oregon, large quantities of organic debris and sediment were deposited on the stream bed. Much of the surface deposit was removed by fall freshets, but a 10% increase in fine sediment was detected in coho salmon (*Oncorhynchus kisutch*) redds during 3 yr following logging (Moring and Lantz 1975). Gravel permeabilities measured at permanent standpipes in this stream were

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reduced to 22–50% of prelogging levels during a 7-yr postlogging period (Moring 1982). In this note, we compare vertical patterns of sediment layering in salmon redds of three streams subjected to clearcut, patch-cut, and no logging.

Methods

Logging in the study area occurred in 1966. One watershed, Needle Branch, was entirely clearcut. The stream channel was cleared of large debris after logging, and the slash was burned in the fall. The Deer Creek watershed was cut in three staggered units and a strip of vegetation was left along the stream, so that large debris did not reach the stream. The control (reference) stream, Flynn Creek, was unlogged. Details of the study area and pattern of logging have been described elsewhere (Hall and Lantz 1969; Brown and Krygier 1971).

Frozen cores were collected from 16 redds during July and August of 1968, following completion of emergence by coho salmon. The sampler was a simplified version of a design by Ryan (1970), which employs dry ice and acetone. Two core samples were taken approximately 1 m apart on the long axis of the redd. We also obtained gravel cores from 11 areas used as redds during the previous year (former redds). The upper 25 cm of each core was split into three equal strata while still frozen using a guillotine-like device. Each sample was then split vertically into two subsamples, one for organic content and the other for gravel size composition. Seven sizes of gravel were determined (<0.83, 1.65, 3.33, 6.35, 12.7, 25.4, and 50.8 mm) based on the McNeil and Ahnell (1964) wet sieving technique. The results of the gravel composition analysis are given in Ringler (1970). In this note, we employed a criterion for "fine sediment" of 3.33 mm, based on Koski's (1966) finding that coho survival was most highly negatively correlated with the amount of bed material below that size.

The percentage of organic material in the gravel samples was determined by weight loss upon ignition. Each stratum was thawed and then dried for 24 h at 105°C. The cooled samples were sieved through a 6.35-mm screen, and two subsamples were burned at 600°C for 5 h in a muffle furnace. Organic debris larger than 6.35 mm occurred infrequently and represented less than 3% of the total organic content by dry weight. Our analysis is therefore based primarily on materials smaller than 6.35 mm.

The statistical tools used in analysis were analysis of variance (ANOVA) and simple linear regression. In the ANOVA's, assumptions of normality and homogeneity for sediment and organic debris were generally met, although there were differences among some of the cell variances. Violation of the assumptions was reduced only slightly with log transformation; the untransformed data were therefore employed throughout our analysis. The data met the assumptions of the simple linear regression model, except that the estimate of variance for the clearcut stream was barely significantly different ($p < 0.05$) from that of the others.

Results and Discussion

Patterns of size distribution among cores were heterogeneous (Fig. 1). In some cases, differences were evident even visually in the pattern or quantity of small gravel in samples 1 m apart in a single redd. On the other hand, relatively small differences were evident in mean sediment content among the three strata (Table 1). A two-way ANOVA (stream × depth) revealed no significant differences among depths ($p > 0.10$), either in redds or former redds. Interaction between stream and depth effects, however, shows that in both logged streams, fine sediment (<3.33 mm) in redds decreased with depth whereas in the reference stream, fine sediment increased with depth. This result was not statistically significant, although it is consistent with the hypothesis that logging may deposit a surface layer of sediment in salmon redds. Brown and Krygier (1971) reported significant increases in suspended sediment in both logged streams. Clearly, our analysis of 8.3-cm depth strata was insensitive to the thinner bands of sediment that were visible in some samples.

Comparing current and former redds shows that in Needle Branch (clearcut watershed) the redds tended to have significantly less sediment than the former redds whereas in Flynn Creek (unlogged) there was somewhat more sediment in the redds than in former redds. Samples of the former redds were not taken from Deer Creek. The interpretation of this outcome is difficult, but the observed interaction in a three-way ANOVA (stream × depth × redd type; $F_{1,93} = 6.96$; $p < 0.01$) confirms that differences among streams masked differences among depths and between redd types.

Within streams, differences among redds were examined with one-way ANOVA of mean sediment content (gravel <3.33 mm). Sediment content differed significantly among redds in both logged streams (Needle Branch, $F_{1,15} = 5.58$, $p < 0.05$; Deer Creek $F_{1,9} = 9.19$, $p < 0.05$) but not in the reference stream where the sample size was smaller. The sediment content of Needle Branch redds averaged 25% lower than in former redds ($F_{1,65} = 8.50$, $p < 0.01$). This difference likely represented the effect of spawning activity by female coho salmon on the intragravel environment. Such an effect of spawning by pink salmon (*O. gorbuscha*) and chum salmon (*O. keta*) has been noted in other streams (McNeil and Ahnell 1964; Cooper 1965; Rukhlov 1969) and for chinook salmon (*O. tshawytscha*) by Lotspeich and Everest (1981). Ringler and Hall (1975) showed that dissolved oxygen in redds of these Oregon streams averaged 15% higher (0.89 mg/L) than in other areas of the stream suitable for spawning.

Organic debris — Organic material, consisting largely of Douglas-fir needles, bark or wood fragments, and amorphous detritus, made up a small fraction of the core samples, the values ranging from 0.33 to 2.85% by weight (Table 2). Nearly all (97%) of the organic debris was small enough to pass through a 6.35-mm sieve.

Although mean differences in organic debris among depths were not significant, the interaction between stream and depth effects was, again, significant ($F_{4,87} = 3.48$, $p < 0.05$). Inspection shows that organic debris in Needle Branch redds was greatest near the surface and decreased with depth but that in Deer and Flynn creeks the smallest amounts of debris were found near the surface, with a general trend toward an increase with depth. Needle Branch was the only logged stream to receive a significant pulse of organic material during logging (Moring and Lantz 1975), and our analysis suggests that the initial increment of organic debris tended to remain near the surface of the gravel. There was no statistically significant difference between Needle Branch redds and former redds in the pattern of distribution of organic debris.

Relationship of organic debris to fine sediment — Organic content was directly related to the quantity of fine sediment (<3.33 mm) in the gravel sample (Fig. 2) ($F_{1,132} = 106.86$, $p < 0.01$), a result that parallels the findings of McNeil and Ahnell (1964) in Alaskan streams. ANOVA revealed no signif-

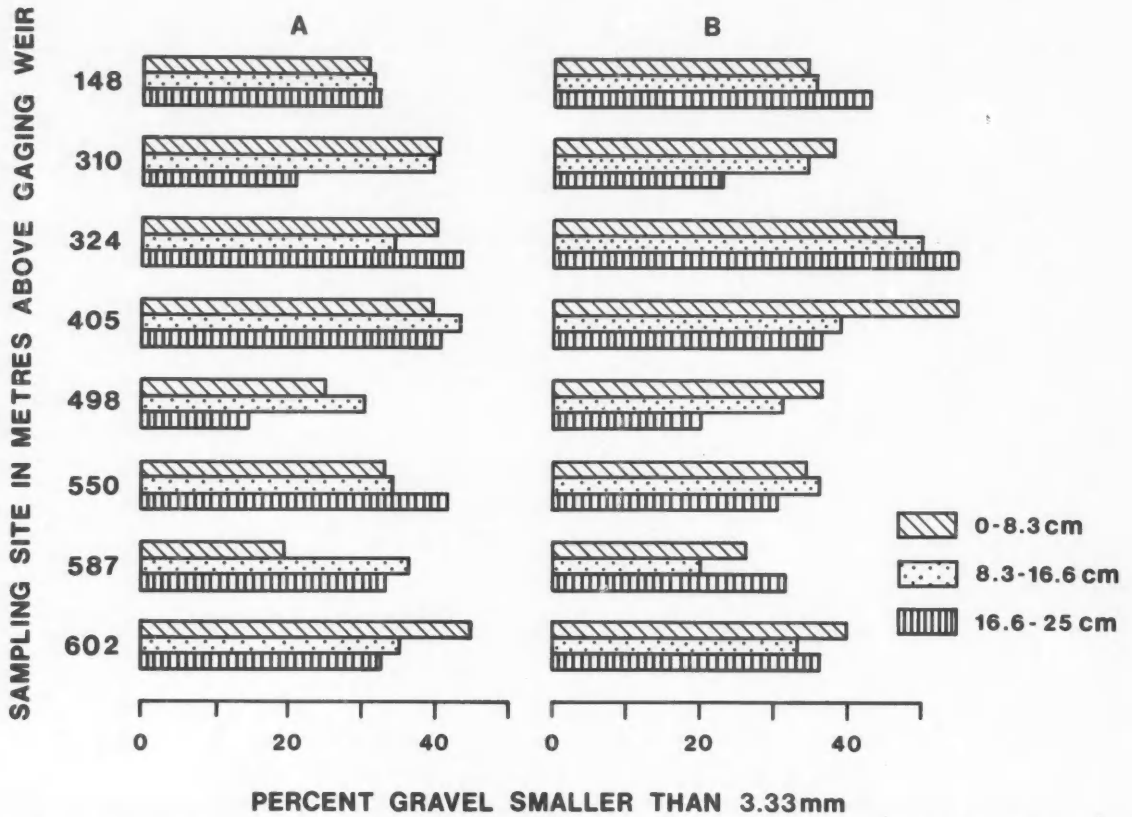

FIG. 1. Vertical distribution of sediment in eight Needle Branch redds. A and B designate the two individual sample sites within each redd, where A is placed 1 m directly upstream of B. Centimetres refer to depth of core.

TABLE 1. Percentage of particles smaller than 3.33 mm in three depth strata of three study streams. Strata: 1 = 0–8.3 cm; 2 = 8.3–16.6 cm; 3 = 16.6–25 cm.

	Stream and treatment												
	Needle Branch, clearcut				Deer Creek, patch-cut				Flynn Creek, reference				
	Mean	Range	No. of redds	No. of samples	Mean	Range	No. of redds	No. of samples	Mean	Range	No. of redds	No. of samples	Grand mean
						Redds							
Stratum													
1	37.4	19.8–56.4	8	16	35.4	27.2–44.1	5	10	41.3	35.6–47.4	3	6	38.0
2	36.1	20.3–51.5	8	16	31.5	22.0–41.5	5	10	44.5	37.5–49.8	3	6	37.4
3	34.2	14.7–56.3	8	16	31.9	23.2–47.0	5	10	49.7	28.9–87.1	3	6	38.6
Avg. or total	35.9			48	32.9			30	45.2			18	38.0
						Former redds							
Stratum													
1	45.0	22.6–87.7	6	6	—	—	—	—	38.1	30.6–46.1	5	7	41.6
2	40.9	24.7–50.2	6	6	—	—	—	—	42.0	28.8–51.5	5	7	41.5
3	49.1	29.7–74.1	6	6	—	—	—	—	44.9	22.6–67.5	5	7	47.0
Avg. or total	45.0			18					41.7			21	43.3
Grand total				66								39	

TABLE 2. Percentage (by weight) of organic material in stratified gravel samples removed from three study streams.

	Stream and treatment												
	Needle Branch, clearcut				Deer Creek, patch-cut				Flynn Creek, reference				
	Mean	Range	No. of redds	No. of samples	Mean	Range	No. of redds	No. of samples	Mean	Range	No. of redds	No. of samples	Grand mean
						Redds							
Stratum													
1	1.67	1.01–2.37	8	16	1.16	0.72–1.49	5	10	1.51	1.28–1.85	3	6	1.45
2	1.55	0.98–2.29	8	16	1.34	1.04–1.83	5	10	1.72	1.24–2.09	3	6	1.54
3	1.21	0.33–2.18	8	16	1.26	0.90–1.72	5	10	1.98	1.19–2.84	3	5	1.48
Avg.	1.48				1.22				1.74				1.49
						Former redds							
Stratum													
1	1.42	0.82–1.68	6	6	—	—	—	—	1.66	1.08–1.80	5	7	1.54
2	1.52	0.76–2.02	6	6	—	—	—	—	1.68	0.85–2.09	5	7	1.60
3	1.55	1.07–2.23	6	6	—	—	—	—	1.56	0.48–2.20	5	7	1.56
Avg.	1.50								1.63				1.57

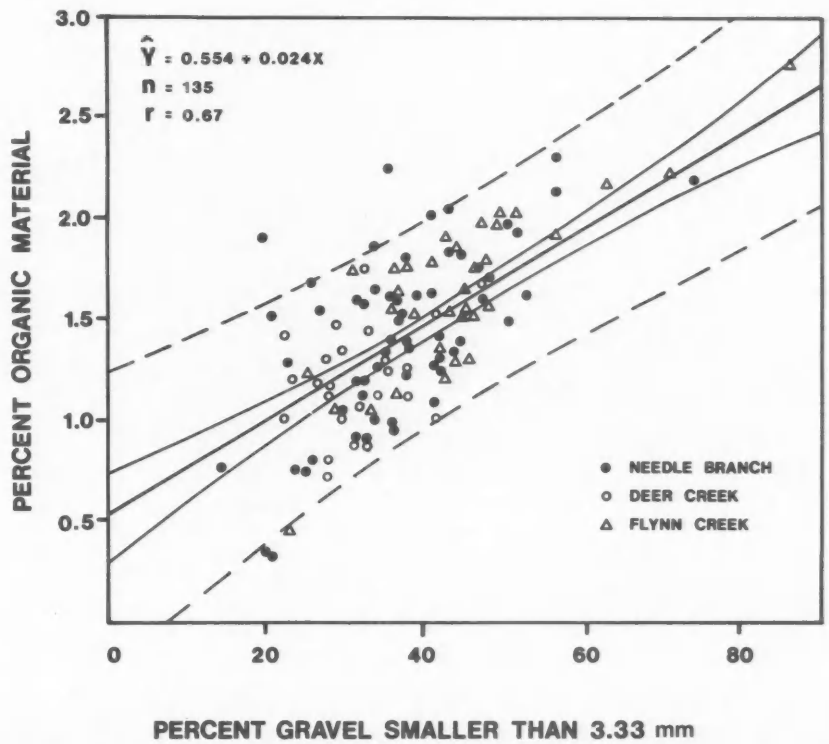

FIG. 2. Relationship between organic debris and sediment in the gravel of the study streams. The solid curved lines represent 95% confidence bands for the regression line; broken lines indicate tolerance bands for an individual future prediction of organic debris.

icant differences among regressions based on data from the three streams, either for the slope or for the regression as a whole ($F_{2,129} = 1.53$, $F_{4,129} = 1.81$). Similarly, the regression equations from redds and former redds did not differ significantly ($F_{1,131} = 1.92$, $F_{2,131} = 1.37$). The percentage of gravel <3.33 mm in these streambeds provided only a rough indication of organic content, since less than half of the variation ($r^2 = 0.445$) in organic debris was explained by variation in sediment content.

Layering of sediment and organic debris often characterizes salmon redds. In Russian streams, sediment was deposited in layers in redds of pink and chum salmon, evidently as a consequence of digging activities of females (Vasilyev 1959; Rukhlov 1969). Everest et al. (1982) reported major differences in gravel composition among sampling sites in Rogue River tributaries, even though the composition of the upper 10 cm was similar. Scrivener and Brownlee (1981) demonstrated variability in gravel composition among depths in Carnation Creek, B.C. Other investigators have designated "fine sediment" as materials <1 mm (Adams and Beschta 1980) or <0.83 mm (Sheridan et al. 1984; Edgington 1984). We found that our data based on analysis of materials <0.83 mm led to the same conclusions as those reported here for materials <3.33 mm.

In our study, gravel composition of coho salmon redds was spatially heterogeneous, but a pattern of layering was related to watershed management practices. In clearcut and patch-cut watersheds, sediment was greatest in the uppermost layer of the redds whereas in the reference stream, the uppermost layer contained the least fine sediment. Furthermore, organic debris in the clearcut stream was greatest near the surface of the redds. Such patterns are consistent with the observed decline of gravel permeability on Needle Branch following logging (Moring 1982) and the inferred reduction in survival to emergence of coho salmon fry (Hall et al. 1987). Spatial variation in composition of spawning gravel may also help explain the extreme range in survival (9–82%) to emergence observed in the long-term Alsea Watershed Study (Moring and Lantz 1975).

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Sustained Exercise Increases Plasma Growth Hormone Concentrations in Two Anadromous Salmonids

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Barrett, B. A., and B. A. McKeown. 1988. Sustained exercise increases plasma growth hormone concentrations in two anadromous salmonids. Can. J. Fish. Aquat. Sci. 45: 747–749.

Sustained exercise for 24 h at 1.5 body lengths per second (bl/s) resulted in a severalfold increase in the plasma growth hormone (GH) concentration of steelhead trout (*Salmo gairdneri*) and coho salmon (*Oncorhynchus kisutch*). Peak levels occurred 10 min postexercise and returned to basal levels within 4 h of exercise termination. GH levels exhibited a steady increase after the fourth hour of exercise and peaked at 24 h. Individuals exercised for 48 h did not exceed plasma GH levels of the 24-h peak.

Un niveau d'exercice soutenu, pendant 24 h et à des déplacements correspondant à 1,5 fois la longueur du corps par seconde, s'est traduit par un accroissement de plusieurs fois la concentration plasmatique de l'hormone de croissance chez la truite arc-en-ciel (*Salmo gairdneri*) et le saumon coho (*Oncorhynchus kisutch*). Les concentrations maximales ont été notées 10 min après la fin de l'exercice et les valeurs étaient revenues à la normale moins de 4 h après la fin de celui-ci. Les concentrations d'hormone de croissance présentaient une augmentation constante après la quatrième heure d'exercice pour atteindre un maximum après 24 h. Les concentrations plasmatiques d'hormone de croissance de poissons dont la durée d'exercice a atteint 48 h n'ont pas dépassé la concentration maximale notée après 24 h.

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Many anadromous salmonids undergo long, arduous migrations both as mature adults returning to the spawning grounds and juveniles leaving freshwater streams for the open ocean (Banks 1969; Brannon et al. 1981). Such migrations entail great energetic costs for the individual, and many anadromous salmonids undergo these migrations without actively feeding en route (Beamish 1964). Hence there is a great demand for the mobilization of stored reserves to facilitate this general increase in metabolic rate. In man, physical exercise is known to induce an increase in plasma growth hormone (GH) concentration (Galbo 1983). Several investigations indicate that GH aids in energy acquisition by stimulating lipolysis through an increase in lipolytic enzyme activity (Harvey et al. 1977; Goodman and Grichting 1983). During sustained swimming the rainbow trout (*Salmo gairdneri*) preferentially mobilizes lipid reserves and conserves body protein (Beamish 1978), and current studies support the lipolytic role of GH when this is injected into coho salmon (*Oncorhynchus kisutch*) parr (Sheridan 1986). We report here on increased levels of plasma GH in response to sustained exercise in the steelhead trout (*S. gairdneri*) and the coho salmon.

Methods

Juvenile steelhead trout (35.6 ± 3.1 g; 16.3 ± 0.7 cm fork length (FL)) and coho salmon (29.1 ± 4.3 g; 15.7 ± 0.4 cm FL) were obtained from a local hatchery and maintained in 120-L flow-through tanks supplied with fresh, dechlorinated water under natural photoperiod and temperature from February to April. Fish were fed once daily to satiation on a local brand of trout and salmon feed.

In experiment A, fish were placed in a recirculating swim tunnel (modified from Brett 1964) and exercised at 1.5 body lengths (bl)/s for 24 h. Control fish were placed in an identical chamber to control for the environment but were not exercised. The tunnel was flushed with fresh, dechlorinated water for each test run and oxygen levels in the chamber never fell below 70% air saturation. Following the 24-h exercise regime, both experimental and control fish were transferred to identical holding tanks and sampled 0, 10, 30, 60, 120, and 240 min postexercise. In experiment B, individuals were exercised at 1.5 bl/s for 0, 1, 2, 4, 6, 12, 24, and 48 h. Fish were bled 10 min postexercise in accordance with peak GH levels as determined in experiment A.

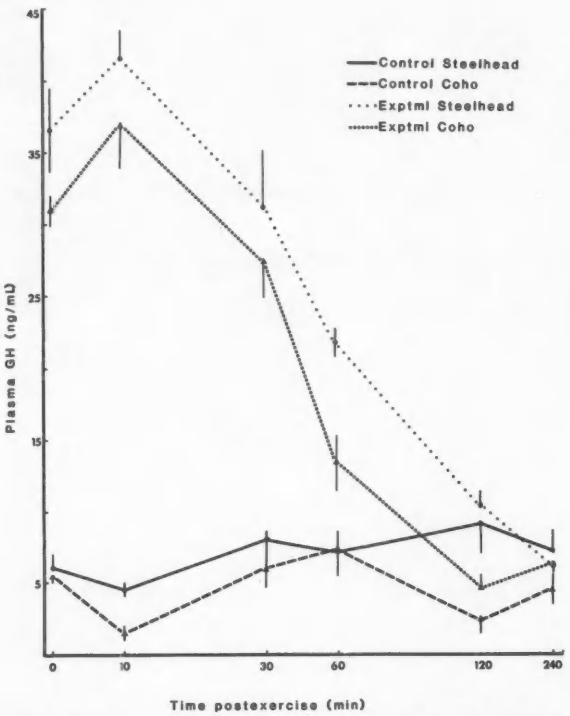

FIG. 1. Levels of plasma GH of juvenile steelhead trout and coho salmon at varying intervals postexercise. Each point represents the mean of eight fish ± SEM.

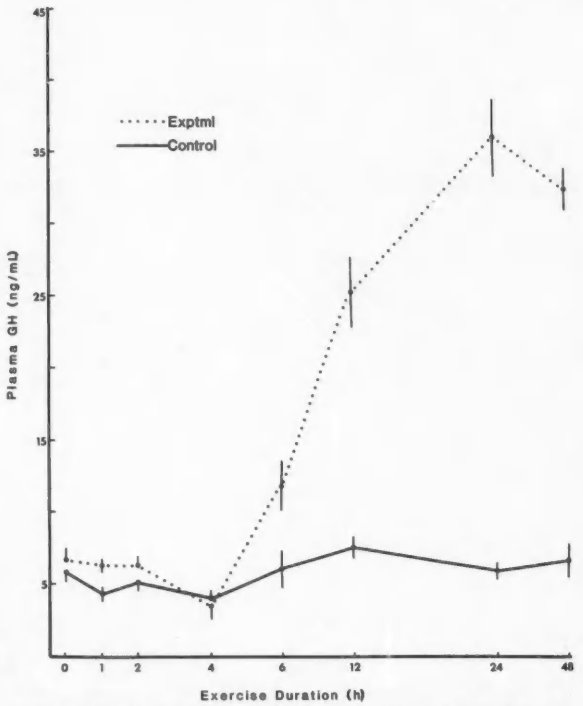

FIG. 2. Plasma GH levels of juvenile steelhead trout 10 min after varying bouts of exercise duration at 1.5 bl/s. Each point is the mean of at least eight fish ± SEM.

Blood was collected via the caudal artery in heparinized capillary tubes. Following centrifugation the plasma was immediately frozen on dry ice and stored at −20°C. Plasma GH levels were determined using an homologous radioimmunoassay (RIA) with coho salmon GH (Wagner and McKeown 1986). Statistical differences between the control and experimental groups were tested using Student's *t*-test.

Results

Sustained exercise dramatically increased plasma GH levels in experimental individuals (Fig. 1). Plasma GH levels peaked at 10 min postexercise (41.3 ± 2.6 ng/mL) and reached levels in excess of 800% as compared with control levels (4.96 ± 1.1 ng/mL). There was no significant difference in peak levels of GH in steelhead trout and coho salmon. Plasma GH concentrations returned to basal levels within 4 h of exercise termination. Experimental individuals exhibited GH levels similar to control animals until the fourth hour of exercise (Fig. 2). Plasma GH concentrations rose to peak levels at 24 h of exercise duration (36.1 ± 3.4 ng/mL). Individuals exercised for 48 h did not achieve GH levels in excess of the 24-h peak.

Discussion

It is well documented that plasma GH levels increase in response to various forms of physical exercise in man (reviewed in Galbo 1983). The results presented here clearly indicate that steelhead trout and coho salmon respond in a similar fashion when required to swim actively for extended periods (Fig. 1). The profile of the GH response following exercise (Fig. 1) is similar to that reported previously in humans (Sutton and Lazarus 1974; VanHelder et al. 1986). Hematocrit levels of exercised individuals (31.8 ± 1.6%) are not significantly different from those of nonexercised control fish (31.0 ± 2.1%) following such bouts of sustained swimming. Thus the increase in GH is not simply an artefact of hemoconcentration.

In humans, this typical GH response has been associated with an increase in lipid mobilization through stimulation of lipolytic enzyme activity (Goodman and Grichting 1983). GH also functions by increasing amino acid incorporation into protein and elevation of the body protein to fat ratio (Martin 1985). Actively swimming fish also require mobilization of stored reserves to facilitate the concomitant increase in metabolic rate (Driedzic and Hochachka 1978). Sheridan (1986) has reported data that support the lipid-mobilizing role of GH in coho salmon. In accordance with human studies, GH was shown to exert its effects through an increase in lipolytic enzyme activity (Sheridan 1986). Furthermore, preliminary results in this laboratory have shown increases in the specific plasma free fatty acids 22:6, 20:4, 18:2, and 16:1 of exercised fish as compared with nonexercised controls (unpubl. obs.).

It is generally accepted that actively swimming fish preferentially mobilize carbohydrates prior to utilization of lipid and protein reserves (Driedzic and Hochachka 1978). This could account for GH levels which do not significantly differ from control individuals over the first 4 h of exercise (Fig. 2). These changes in GH, however, could also be an effect of different changes in various GH pools such as pituitary, blood, and other tissues. It should also be noted that these experiments were

conducted in late winter with water temperatures between 4 and 6°C. Subsequent work in this laboratory has shown a seasonal difference in basal GH levels (B. A. Barrett and B. A. McKeown, unpubl. data). Individuals maintained in low temperatures (4–6°C) exhibit significantly lower plasma GH levels (2–10 ng/mL) than fish which are held at higher temperatures (15°C; 30–40 ng/mL). It is possible that this low temperature may be another reason for the delay in GH response during exercise (Fig. 2).

Leon (1986) has recently shown that brook trout (*Salvelinus fontinalis*) reared under conditions of sustained swimming (1.5–2.0 bl/s) grow faster than unexercised fish. Over a 10-wk period the exercised individuals gained more weight, were able to consume more feed, exhibited a better food conversion rate, and showed increased stamina. Similar data have also been reported in salmon (Kuipers 1982). Certainly the increase in stamina could be directly attributable to exercise acclimation. However, by analogy with human GH physiology and the exercise metabolism of fish presently known, it is quite possible that the results of Leon's exercise study are all attributable, in some degree, to the increase in plasma GH levels that we report here.

Acknowledgements

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Behavioral Alterations in Arctic Char (*Salvelinus alpinus*) Briefly Exposed to Sublethal Chlorine Levels

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Jones, K. A., and T. J. Hara. 1988. Behavioral alterations in Arctic char (*Salvelinus alpinus*) briefly exposed to sublethal chlorine levels. Can. J. Fish. Aquat. Sci. 45: 749–753.

Changes in the locomotor behavior of Arctic char (*Salvelinus alpinus*) exposed inadvertently to sublethal chlorine concentrations (>19 μg total residual chlorine/L) for 6 d are described. Compared with their preexposure behavior, the char became hypoactive, much more strongly thigmotactic, and less responsive to a food extract during the exposure. After the high chlorine levels subsided, the char continued to be hypoactive for at least 1 wk and failed to respond positively to the food extract for 20 d. There is evidence that some individuals displayed chlorine stress behavior for at least 46 d after the return of nonstressful conditions.

Les auteurs décrivent les modifications du comportement locomoteur d'ombles arctiques (*Salvelinus alpinus*) exposés par inadvertance à des concentrations sublétales de chlore (>19 μg de chlore résiduel total par litre) pendant 6 j. Comparativement à leur comportement avant l'exposition, les ombles sont devenus, pendant la durée de l'exposition, hypoactifs et beaucoup plus thigmotactiques, ceci en plus de moins réagir à un extrait alimentaire. Après la chute des fortes concentrations de chlore, les ombles ont continué d'être hypoactifs pendant au moins 1 sem et n'ont pas réagi à l'extrait alimentaire avant 20 j. Selon certains indices, des individus ont continué à présenter un comportement de stress dû au chlore pendant au moins 46 j après le retour aux conditions normales.

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Chlorine is recognized as a potentially harmful substance for fish. Toxicity levels have been established for a variety of species (U.S. EPA 1984) and several studies have shown that fish orient negatively to chlorine gradients when refuge is available (see reviews by Cherry and Cairns 1982; Brown et al. 1982; Giattina and Garton 1983). However, refuge is not always available, and at times, fish are required to endure high chlorine levels. The end results of lethal exposures are obvious, but other than the studies by Arthur and Eaton (1971) and Dandy (1972), little attention has been paid to the possible deleterious effects of sublethal chlorine exposures, this despite the fact that generally more fish will be exposed to sublethal than lethal concentrations following a pollution incident (Klerekoper 1976). Here we describe behavioral alterations in a group of Arctic char (*Salvelinus alpinus*) inadvertently exposed to sublethal levels of chlorine lasting several days.

Materials and Methods

The Arctic char were obtained from the Freshwater Institute's Experimental Fish Hatchery in Gunton, Manitoba. They ranged 10–17 cm in total length and weighed 35–50 g. The fish were originally intended for a study on acidification (Jones et al. 1987), and for that purpose, 50 char were set apart as controls in two 56-L glass aquaria supplied with a flow of laboratory water at a minimum daily rate of 2 L/g fish (Winnipeg city water dechlorinated by activated charcoal filtration and ultraviolet radiation; 10–12.5°C, pH 7.8, hardness = 0.78 mmol $CaCO_3$/L). Other aspects of the holding conditions including water quality are detailed in Jones et al. (1985). The acidification study was intended as a long-term experiment with the controls tested intermittently throughout. Each fish was tested singly and only once within a Plexiglas behavior trough (200 × 33 × 16 cm) supplied with a flow through of laboratory water (6 L/min) and divided longitudinally by perforated PVC into three sections: an influent side, a central swimming channel, and an effluent side (Jones and Hara 1985). The construction of the influent side was such that a localized infusion of food extract (Martin's Trout Feed) generated a stationary field, or "plume," of treated water in the middle of the central channel. Testing began on September 9, 1984 (day 0), when seven control fish were monitored for their locomotor performance in the trough. Nine more fish were tested in the same manner on day 24. A test consisted of transferring a char from the holding aquaria to the central channel, allowing 30 min for acclimation, and then electronically monitoring the subject's unit position (0–100) along the length of the trough as it swam freely about for two 8-min periods: one immediately before and one during the introduction of the extract. The subject was then removed and the trough prepared for the next test. The data (unit position versus time) for each monitoring were stored on computer disk and three behavioral parameters analyzed: (1) the unit distance traveled during the initial 8-min period (one length of the trough equals 100 units); (2) the amount of time accumulated at the two ends of the trough (unit areas 0–10 and 90–100) during this same period; and (3) time accumulated in the central plume of food extract (unit area 35–55) during the second 8-min period. The three parameters were taken to be measures of activity, thigmotaxis, and attraction to a chemical feeding stimulant, respectively.

Beginning on day 34 and continuing through day 39, the municipal waterworks increased the chlorine in city water to levels exceeding the handling capacity of the dechlorination system. Consequently, chlorine levels in the aquaria influents, as measured by the orthotolidine colorimetric method (Taras et al. 1976), exceeded 19 μg total residual chlorine (TRC)/L for 6 d. Actual levels within the aquaria were not monitored but were assumed to be slightly lower due to the residence time and aeration within the aquaria. None of the control fish died during this period, but the original acidification study was terminated due to the incident. Nevertheless, we continued monitoring the behavior of the controls during and after the chlorine surge. Five more fish were monitored for their locomotor behavior on day 39, the last day of the surge. Following the exposure, seven fish were monitored on day 46, and five fish were monitored on each of days 59 and 85. Our objective was to describe the behavioral changes resulting from the chlorine exposure and to determine if and when the char recovered normal behavior patterns.

Measurements on the three locomotor parameters were statistically analyzed by first comparing the two presurge means (days 0 and 24) with a Student *t*-test (nonpaired observations, equal variances, two-tailed). No significant difference was found between the two groups in any of the parameters. Therefore, the measurements for the two days were pooled to provide a stronger statistical control and then compared with the remaining daily means (days 39, 46, 59, and 85) with a one-way analysis of variance. All values were logged before analysis to achieve homogeneity of variance (although, in fact, the results remain the same), and statistical significance in the following graphs were indicated on this basis. However, the original arithmetic values and their means were actually plotted for easier interpretation by the reader.

It must be emphasized that there are no formal controls for this study. Hence, it might be argued that any changes in locomotor behavior may actually be the result of random or seasonal variation; i.e. repeated measurements on these three parameters over time could have yielded means of similar values even without the influence of chlorine. To test for this, we analyzed the records of 234 fish that had served as controls in prior experiments during March–August 1982 and February–April 1983. All 234 fish were used to measure the variation in activity and thigmotaxis, but only 54 could be used to assess the variation in food extract attraction, since not all of the fish were tested with this stimulus. We divided the fish into groups of six (the median size of a daily test group) in the order of their testing, and the means of the three parameters were calculated for each group. These means have been plotted in the above-mentioned graphs for comparison.

Results and Discussion

Between days 0 and 33, chlorine levels in the aquaria influents remained at or below 10 μg/L (Fig. 1A). Although the levels between days 24 and 33 were higher than desired, we observed no instances of abnormal behavior by the fish during the first 33 d. While in the aquaria the char were active, occasionally exhibited aggressive social interactions, and readily accepted food. Moreover, those individuals tested on days 0 and 24 showed the behavioral patterns typical of nonstressed fish: a brief initial period of quiescence followed by the subject actively exploring the trough, and a strong localization on the chemical field when the food extract was introduced (Jones et al. 1985). By pooling the data for days 0 and 24, the average preexposure char could be characterized as moderately active ($\bar{X}$ = 2359 units traveled in the alloted 8 min), slightly thig-

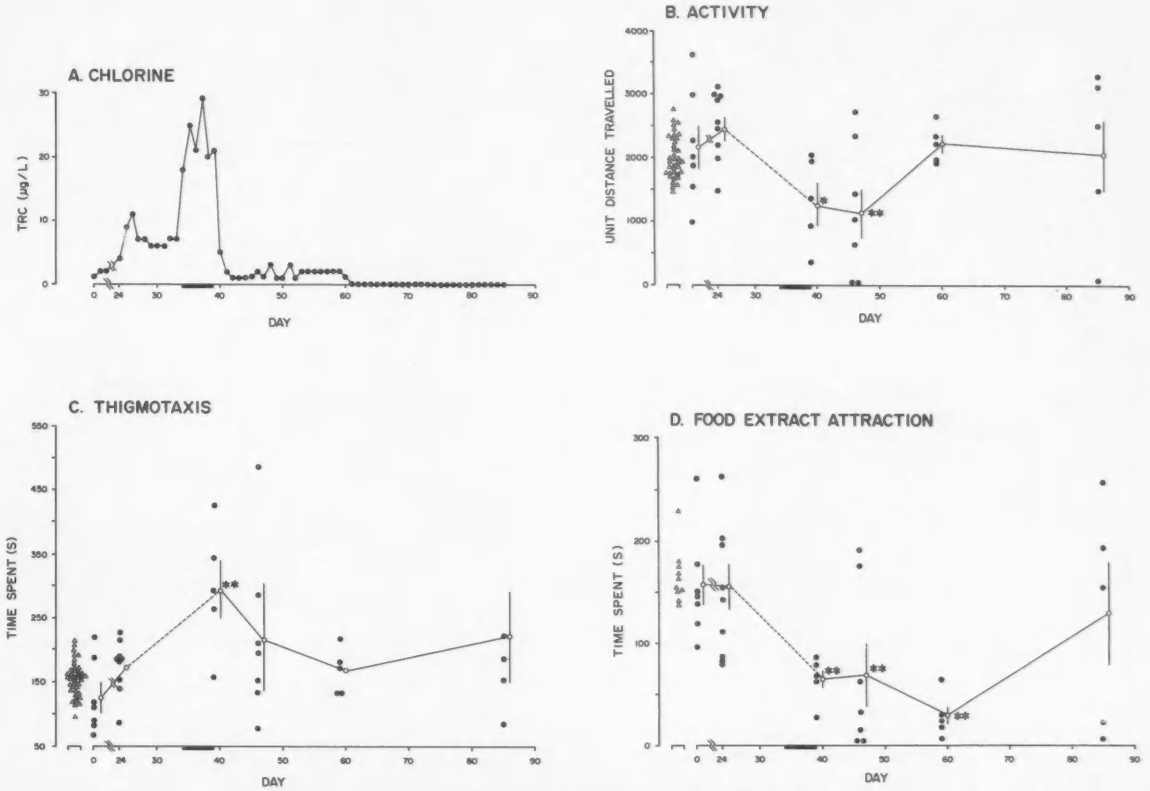

FIG. 1. Levels of (A) chlorine, (B) activity, (C) thigmotaxis, and (D) food extract attraction during the period of observation. The heavy line on each graph indicates the time of the chlorine surge. Solid circles show actual values; open circles show means (± 1 SE). Asterisks indicate means significantly different (one, $p \leq 0.05$; two, $p \leq 0.01$) from the pooled presurge means (days 0 and 24). Triangles show the means ($N = 6$) calculated from the records of 234 fish used as controls in prior experiments.

motactic ($\bar{X}$ = 152 s spent at the two ends of the trough), and highly attracted to the food extract ($\bar{X}$ = 154 s spent in the chemical field).

Beginning on day 34, chlorine levels in the influents rose sharply due to the excessive chlorine content in the incoming city water. The levels then remained elevated above 19 µg/L through day 39, peaking on day 37 at 29 µg/L (Fig. 1A). During this time the char in the aquaria became noticeably lethargic, displayed little interest in food, and were often found lying on the bottom huddled in a corner. When we tested these fish on day 39, the average char was 43% less active ($\bar{X}$ = 1339 units), 95% more thigmotactic ($\bar{X}$ = 297 s), and spent 58% less time localizing the food extract ($\bar{X}$ = 64 s) relative to the char tested before the chlorine surge (Fig. 1B–1D).

The means of the fish tested on day 39 lay outside the distribution of the means calculated for the 234 (or 54) control fish of earlier experiments in all three parameters (Fig. 1B–1D). Moreover, when the control means were plotted against time of year (not shown), there was no indication that any of the three parameters normally gravitate towards the levels recorded on day 39, the scatter of the means being essentially random over the 7 mo (February–August) considered. Thus, although the fall months were not taken into account, there is no evidence that the behavioral changes observed during the chlorine surge resulted from random or seasonal variation.

The time course of recovery from the chlorine surge could be traced through the fish tested on days 46, 59, and 85. Recovery time differed for each of the three locomotor parameters. Thigmotaxis had largely recovered by day 46, but at least one fish on each of days 46 and 85 showed an unusually strong thigmotaxis. Activity remained significantly depressed on day 46, five fish having values lying well below the presurge pooled mean. By day 59 the majority of the fish tested showed no effects in this parameter, but one fish was still very hypoactive on day 85. Attraction to the food extract, the last of the three parameters to recover, remained sharply depressed 20 d after the surge (day 59). Three of the five fish tested on day 85 showed normal responses, and the mean for that day was not statistically different from that of the pooled presurge fish. Nevertheless, even by this late date, two fish clearly failed to respond positively to the food extract. As noted above, one of these fish was also strongly thigmotactic and quite hypoactive.

The results provide evidence that a sublethal exposure to chlorine alters Arctic char locomotor behavior and that the effects on behavior are chronic, here a minimum of 46 d for some individuals. It might be predicted, then, that brief sublethal chlorine exposures will have long-term adverse effects on salmonids in natural settings. However, two issues need to be resolved before the ecological significance of these results can be understood. First, chlorine avoidance thresholds for the

various salmonids need to be more firmly established. The avoidance thresholds shown by rainbow trout (*Salmo gairdneri*) and coho salmon (*Oncorhynchus kisutch*) in steep gradients have been reported to be as low as 1–2 μg/L and as high as 50–100 μg/L (Sprague and Drury 1969; Stober et al. 1980; Cherry et al. 1982). Whereas even the higher values for threshold avoidance are generally believed to permit salmonids to avoid lethal concentrations — ranging from a mean LC50 of 62 μg/L for rainbow trout to 117 μg/L for brook trout (*Salvelinus fontinalis*) (U.S. EPA 1984) — the discrepancy between the reported avoidance thresholds becomes crucial when considering sublethal effects. Specifically, if the lower values for the avoidance thresholds are correct, then salmonids would seem capable of avoiding, if possible, even the sublethal concentrations described here; if the higher values are correct, they would not. Under natural settings the higher threshold values may more closely approximate reality, since fish will tolerate substantially higher levels of chlorine when such factors as food and preferred temperatures are present as well (Hose and Stoffel 1980; Cherry et al. 1982).

The second issue concerns the method of chlorine presentation. Among its other applications, chlorine is used to disinfect effluents and, in thermal discharges, as an antifowling agent. In both cases it is released intermittently (Grieve et al. 1978; Cherry and Cairns 1982). Fish are more resistant to repetitive pulses of lethal chlorine concentrations than to continuous exposures at the same level (Brooks and Seegert 1977). Whether this holds true for sublethal effects needs to be determined, as does the possibility of interactions with temperature (Heath 1977). It would be particularly helpful to know the rate at which sublethal effects accumulate, from which critical exposures could be established.

At present the results presented here are likely more relevant to those workers maintaining fish on dechlorinated water. Clearly, mere survival of experimental subjects to high chlorine levels, even if only acute, is not enough to permit continued confidence in their fitness. Sublethal effects could have subtle long-term consequences for ongoing experiments.

It is noteworthy that these same behavioral changes in Arctic char can be induced by prolonged exposure to sublethal levels of sulfuric acid (Jones et al. 1985). Such a similarity between sulfuric acid and chlorine in their effects on behavior may well be explained by the facts that hypochlorous acid is apparently the most harmful fraction of chlorine in water (Cherry et al. 1977) and that the causative agent of acid stress is probably the cation (Ellgaard and Gilmore 1984). It is known that the surficial tissues of fish are readily damaged by both sulfuric acid and chlorine (Daye and Garside 1976; Bass et al. 1977), thereby providing common histological and physiological avenues through which the two chemicals can affect the same behaviors.

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ANNOUNCEMENTS/AVIS

First Announcement

Interdisciplinary Conference on Natural Resource Modelling and Analysis

September 29 – October 1, 1988
Halifax, Nova Scotia, Canada
Saint Mary's University
and
Bedford Institute of Oceanography
(Co-sponsored by the Resource Modelling Association)

This conference will focus on the development and use of quantitative models to analyse biological, economic, social, and technical aspects of fisheries, forestry, and other renewable resource industries. It will be of interest to those in industry, government, universities, resource management agencies, and other organizations whose work involves, or could benefit from, the quantitative analysis of natural resource problems. The conference will provide a forum for the sharing of methodologies and modelling approaches across traditional discipline boundaries. Plans for the conference include (1) invited speakers representing a variety of backgrounds, geographical regions, and areas of application, (2) contributed paper sessions on (i) complexity and uncertainty in resource systems, (ii) new methods for resource management, and (iii) natural resources/human resources, (3) a workshop on resource industry regulation and the role of models, and (4) a panel discussion with industry and government representatives.

Those interested in presenting a paper at the conference are invited to submit a 1-page abstract; *deadline: June 30, 1988*. For further information, plese contact **Dr. Tony Charles**, Finance and Management Science, Saint Mary's University, Halifax, N.S. B3H 3C3, Canada (telephone (902) 420-5732), or **Dr. George White**, Modelling and Statistics Group, Biological Sciences Branch, Bedford Institute of Oceanography, P.O. Box 1006, Halifax, N.S. B2Y 4A2, Canada.

Première annonce

Conférence interdisciplinaire sur la modellisation et l'analyse des ressources naturelles

29 septembre au 1 octobre 1988
Halifax (Nouvelle-Écosse)
Saint Mary's University
et
l'Institute d'océanographique de Bedford
(Co-parrainée par la Resource Modelling Association)

La présente conférence sera centrée sur l'élaboration et l'utilisation de modèles quantitatifs pour l'analyse des aspects biologiques, économiques, sociaux et techniques de la pêche, de l'exploitation forestière et d'autres industries exploitant des ressources renouvelables. Elle portera un intérêt particulier pour les personnes qui, dans le cadre de leurs travaux au sein des industries, des gouvernements, des universités, des organismes de gestion des ressources et d'autres organisations, doivent effectuer des analyses quantitatives de la dynamique des ressources naturelles. La conférence agira aussi comme enceinte pour l'échange de méthodes et de techniques de modélisation qui traversera les frontières disciplinaires traditionnelles. On planifie les activités suivantes : 1) des présentations par des conférenciers invités de diverses formations et régions géographiques travaillant dans divers domaines d'application; 2) des séances de présentation d'études sur les thèmes suivants : i) la complexité et l'incertitude liées aux ressources, ii) les nouvelles méthodes de gestion des ressources et iii) les ressources naturelles et humaines; 3) un atelier sur la réglementation des industries d'exploitation des ressources et le rôle de modèles; et 4) une réunion-débat avec des représentants des industries et des gouvernements.

Les personnes intéressées à faire une présentation au cours de la conférence doivent envoyer un résumé d'une page, *au plus tard le 30 juin 1988*, à une des personnes suivantes dont on peut aussi obtenir d'autres informations : **Dr Tony Charles**, Finance and Management Science, Saint Mary's University, Halifax (N.S.) B3H 3C3, Canada (téléphone : (902) 420-5732), ou **Dr George White**, Modelling and Statistics Group, Biological Statistics Branch, Bedford Institute of Oceanography, P.O. Box 1006, Halifax (N.S.) B2Y 4A2, Canada.

Canadian Conference for Fisheries Research (CCFFR)

Quebec City, Quebec January 4–5, 1989
Announcement of Conference and Call for Papers

This conference will be held at the Quebec City Aquarium, January 4 and 5, 1989. The major themes of this conference will be (1) measuring the health of fisheries habitats, (2) oceanographic aspects of aquaculture, and (3) environmental control of freshwater and marine production in relation to fisheries.

While papers on other topics may be submitted, authors are

Conférence canadienne sur la recherche des pêches (CCRP)

Québec (Québec), les 4 et 5 janvier 1989
Avis de conférence et demande de résumés

Cette conférence aura lieu les 4 et 5 janvier 1989 à l'aquarium de Québec. La conférence portera sur les principaux thèmes suivants : 1) la mesure de l'état de santé de l'habitat du poisson; 2) les aspects océanographiques de l'aquiculture; et 3) le contrôle environnemental de la production en eaux douces et marines, en rapport avec les pêches.

encouraged to adhere to these themes as much as possible.

Deadline for abstracts is August 30, 1988. They should be typed (maximum 200 words, single spaced) and the type of presentation (oral/poster) indicated.

Abstracts should be addressed to Dr. Louis Fortier, GIROQ, Department of Biology, Laval University, St. Foy, Que. G1K 7P4.

On peut présenter des études sur d'autres sujets quoiqu'on encourage les chercheurs à s'en tenir autant que possible à ces thèmes.

La date limite d'envoi de résumés a été fixée au 30 août 1988. Le résumé doit être dactylographié (200 mots au maximum, simple interligne) et l'on doit préciser le genre de présentation (prés. orale/affiche).

Veuillez faire parvenir votre résumé à M. Louis Fortier, D.Ph., GIROQ, Département de biologie, Université de Laval, Ste-Foy (Qué) G1K 7P4.

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